DEVELOPMENT OF A NEW ROUTE FOR DIRECT CONVERSION OF WET ALGAE TO BIODIESEL

by

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Abstract

Algae have been studied as a potential biodiesel feedstock by identifying on a global scale suitable cultivation locations for three specific cases (EU, US and Brazil) based on the area requirements.

A direct conversion of oil harvested from wet algae to biodiesel was undertaken using ethanol at supercritical conditions, eliminating the use of catalyst, feedstock drying and the oil extraction steps.

Chlorella vulgaris with 7.3% wt. lipid content was characterised (by elemental, chemical and thermal analyses) and used to assess the supercritical ethanol approach. A biodiesel yield of 47.5% wt. was achieved in a flow reactor at 260°C, 75 bar, aqueous algae concentration of 6 $mg \cdot mL^{-1}$ and 2 $mL \cdot min^{-1}$ flowrate. This result demonstrates the advantages of the flow reactor over a batch process where the maximum biodiesel yield was 26% wt. after 6 hours.

A life cycle analysis of the proposed route showed that biodiesel yield must exceed 60% wt. to make the process competitive when compared to the traditional route of oil extraction and catalyst transesterification adopted to algae biodiesel production. In comparison to the soybean biodiesel, the use of algae as feedstock would not be justified unless improvements to reduce energy consumption are made.

To my family…

Mario, Shirlei and Vitor.

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Publications

Assessment of algae biodiesel viability based on the area requirement in the European Union, United States and Brazil

- Renewable Energy (Impact Factor = 3.476)
- http://dx.doi.org/10.1016/j.renene.2014.12.059

Protein Extraction from Microalgae using Ultrasound

- Under Review

Direct Conversion of Wet Algae to Biodiesel in Supercritical Ethanol

- Under Review

Conferences

University of Birmingham Research Poster Conference, 2014 (poster)

- Runner-up Prize of College of Engineering and Physical Sciences
- Assessment of Algae Biodiesel Viability Based on Area Requirement
- https://intranet.birmingham.ac.uk/as/studentservices/graduateschool/documents/public/rpc/RPC2014winners/laisperanza.pdf

22nd European Biomass Conference and Exhibition, 2014 (poster)

- Area Assessment for Algae Biodiesel Production in the EU, the US, and Brazil

VIII ABEP Conference, 2016 (oral presentation)

- Third Generation Biodiesel Production in Supercritical Ethanol

UK Algae Conference, 2016 (poster and flash presentation)

- Direct Biodiesel production from Algae

6th International Symposium on Energy Challenges & Mechanics (ISECM) – towards a big picture, 2016 (oral presentation)

- Direct biodiesel production from Algae in Supercritical Ethanol
- http://www.nscj.co.uk/ecm6/sessions/A06_183.pdf

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List of Abbreviations

B20	Diesel blend with 20% biodiesel
BBM	Bold Basil Media
BBMAlgae	Algae cultivated in BBM media
BSA	Bovine Serum Albumin
C	Carbon
CCD	Central Composite Design
CT	Catalyst Transesterification
DCW	Dry Cell Weight
DoE	Design of Experiments
E25	Gasoline blend with 25% ethanol
EU	European Union (27 countries)
f/2Algae	Algae cultivated in f/2 media
FAEE	Fatty Acid Ethyl Ester
FAME	Fatty Acid Methyl Ester
FFA	Free Fat Acid
FID	Flame Ionization Detector
GC	Gas Chromatography
GHG	Greenhouse Gases
GIS	Geographic Information System
GWP	Global warming potential
H	Hydrogen
HHV	High Heat Value
IEA	International Energy Agency
IS	Internal Standard (methyl heptadecanoate)
ISO	International Organization for Standardization
LCA	Life Cycle Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LS	Lysis Solution
MS	Mass Spectrometry
N	Nitrogen
NER	Net Energy Ration
O	Oxygen
OECD	Organisation for Economic Co-operation and Development
OP	Open Pond
PBR	Photobioreactors
PNPB	Biodiesel Production and Usage
SCDT	Supercritical Direct Transesterification
SC-EtOH	Supercritical Ethanol
SEM	Scanning Electron Microscope
TG	Triacylglycerol
TGA	Thermogravimetric Analysis
UK	United Kingdom
Us	Ultrasonicator
US	United States

1. Introduction

1.1. Motivation

Currently the world uses a large volume of fossil fuels which creates environmental problems, such as increasing global warming. Diesel consumption is approximately 1,450 billion litres per year, which represents a direct emission of 3,886 billion kg of CO_2 and equates to 12% of the world's CO_2 emissions (EIA, 2010).

Based on this, and driven by the growing debate over environmental and economic impacts of energy sources, the production of alternative liquid fuels is strategically important. Alternative fuels can be used to substitute fossil-fuels contributing to improved sustainability, which is a world target as discussed in the United Nations Framework Convention on Climate Change through the COP 21 and 22 (Conference of the Parties) (COP, 2016). UK, per example, uses a system of carbon budgets to restrict the total amount of emitted greenhouse gases over a 5 year period, so if there is a rise in emissions in one sector, there should be a fall in another (BEIS, 2016). In 2016, the carbon budge required a 52% reduction in emissions by 2025 and the power sector has an important part in this target (Thompson, 2016).

Biodiesel is a feasible option for replacing diesel since its substitution does not require substantial engine modifications. This is different to bioethanol which requires engine modification (flex fuels cars). Biodiesel also has advantages over

other alternative fuels, such as hydrogen, which need a complete new market structure. Another fuel with the same potential of replacement is biobutanol for gasoline substitute.

Biodiesel is defined as a fuel made up of monoalkyl esters of long-chain fatty acids derived from bio-oils, usually fatty acid methyl esters (FAME) or fatty acid ethyl ester (FAEE) (ASTM, 2012) and can be produced from vegetable oil by transesterification of the triglycerides or esterification of fatty acids. The chemical reactions of FAEE production (by the reaction with ethanol) are presented in Figure 1.1.

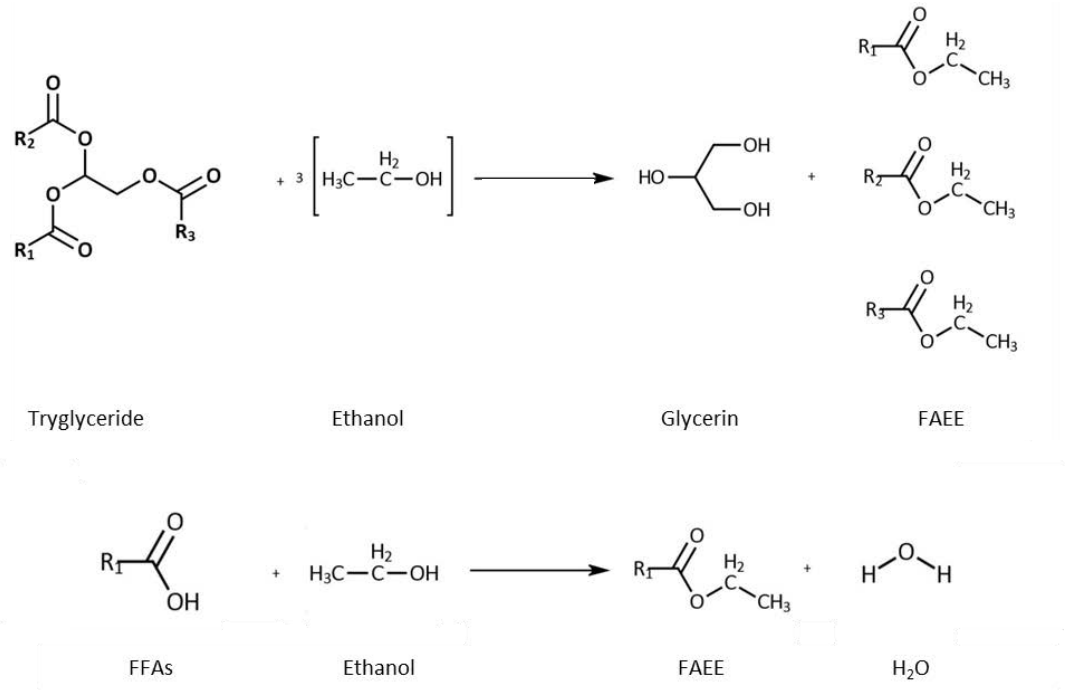

Figure 1.1. FAEE production reactions

Depending on the raw materials used and the production technology, biodiesel can be divided into three generations. The first generation is represented by fuels produced from biomass that can also be used as food, such

as rapeseed and soybeans; second generation is from non-food crops such as *Jatropha curcas,* and waste oil; and third generation is from algae and sea weed biomass (Abdelaziz et al., 2013a, Nigam and Singh, 2011).

The advantages of third generation biodiesel over the other two include:

- High lipid content of algae;
- Fast growth cycle;
- High productivity per area;
- Capability of recycling waste CO_2 emissions; and
- Ability to grow in non-arable and non-productive land.

Biodiesel production from algae can be divided into six main steps: feedstock cultivation; harvesting; feedstock processing (drying); oil extraction; biodiesel production; and fuel refining.

One important identified limitation in the use of algae for fuel production is the energy requirement in the necessary pre-treatment steps of drying and oil extraction and also the separation of the catalyst from the final product. One possible route to decrease the energy demand during the FAEE production is to eliminate the drying and oil extraction steps by doing a direct conversion of wet algae to biodiesel using ethanol in supercritical conditions. This route has been previously tested in a batch reactor with dry algae biomass (Reddy et al., 2014) and in a flow reactor with vegetable oil (Velez et al., 2012). It is therefore possible to presume that this would work in a flow reactor with algae biomass. *Chlorella*

vulgaris was chosen based on its availability and fast growth rate at the University of Birmingham.

The use of supercritical alcohol increases the reaction rate by increasing the miscibility of the oil and solvent and decreasing mass-transfer limitations (Silva and Oliveira, 2014), with ethanol being used because it is a renewable solvent. It was hypothesised that other parameters; such as temperature, water content of the feedstock, pressure and retention time could also influence the biodiesel. The use of alcohol (in this case ethanol) in direct transesterification of wet algae was investigated to avoid drying.

In conjunction with the above, this PhD project investigated the production of third generation biodiesel, through the study of the involved policies. Three specific cases were compared – European Union (EU), United States (US) and Brazil. The policies in these countries were chosen to represent different approaches from around the world.

1.2. Aim and objectives

The aim of this project was to assess a new route for biodiesel production from algal biomass through direct transesterification with supercritical ethanol in order to produce a more environmentally friendly and feasible route for biodiesel production. The follow objectives were proposed to achieve this aim:

- Understand the biodiesel market and the role algae might play in it;

- Assess the potential to produce biodiesel from algae based on the area requirement for its production;
- Select and characterise an algae strain, understanding the important parameters and techniques involved for application in the direct transesterification route;
- Develop a new route for continuous direct transesterification (using supercritical ethanol) and test in a flow system;
- Make an energy balance and environmental assessment in order to verify the technical feasibility of the direct transesterification in supercritical ethanol.

1.3. Thesis structure

This thesis is structured with seven main chapters. The first is the introduction giving an overview, the motivation and the objectives of this study (Chapter 1). Chapter 2 presents a literature review with the current knowledge and gaps in the research. Chapter 3 covers the biodiesel market structure and the potential of algae as feedstock for fuel production. Chapters 4 and 5 present the laboratory work completed to develop the new route of biodiesel production; in Chapter 4 the alga is characterised, while Chapter 5 presents the results of direct supercritical transesterification. Chapter 6 concludes the analysis of the new route by environmental analysis coupled with an energy balance of the system. The thesis is completed with a chapter of conclusions and proposals for future work (Chapter 7).

2. Literature Review

2.1. Overview

This literature review covers the total production of biodiesel. Firstly the biofuels panorama and the importance of biodiesel are presented, followed by the analysis of available feedstocks, the use of algae, its potential as feedstock for biodiesel production, available technologies for this process and its life cycle analysis.

2.2. Biofuels panorama

The search for new renewable energy sources, including biofuels, has been driven largely by the rise in world energy demand and the enlarged debate about environmental concerns involving the increase in emissions of greenhouse gases (GHG) and consequently global climate change. New solutions need to address three main criteria for sustainability: the availability to replace petroleum fuels in the long term (renewable source); the emission of low or none GHG pollutants (environmentally friendly); and the economic and technological feasibility at large scale (Wen et al., 2009).

Biofuels are renewable fuels for transportation in liquid or gaseous form produced from biomass. The main biofuels are bioethanol, biodiesel, biobutanol, syngas and bio-hydrogen. They are potentially carbon neutral (CO_2 emissions during the combustion are similar to the capture during the feedstock production)

and they contribute to agricultural market development with the use of different feedstocks for different climate conditions.

The biofuels market today competes with very low priced fossil fuels and is not helped by the price of biofuel feedstock which makes the biofuels more expensive (Wen et al., 2009). Third generation biomass, technology development (such as supercritical fluid use), governmental policies of taxation and incentives could lead to reductions in this cost and make biodiesel an attractive alternative. The governmental actions to incentivise biofuels and the technology for third generation biodiesel production are presented in sections below.

Biodiesel is able to directly replace fossil diesel without significant changes in transport technology and fuel distribution. It therefore has the potential for a fast implementation. Biodiesel was first globally produced and commercialised in the 1990s and the production has been increasing since then (Balat, 2009).

2.3. Biodiesel production

Biodiesel (fatty acid methyl or ethyl ester – FAME or FAEE) is produced by the transesterification and esterification of glycerides and free fatty acids (FFA) from bio derived oils (vegetable oil or animal fat) (Ma and Hanna, 1999). The first patent for biodiesel production from vegetable oils is from the 1940s (Ma and Hanna, 1999). Since it implantation, the process principles have not changed significantly on the large scale, where the biodiesel is produced with a alkaline catalyst in batch or flow reactors (Balat, 2009). An example of an industrial

process utilising catalytic transesterification in a batch reactor is presented in Figure 2.1. The process starts with the addition of oil, alcohol and catalyst to the reactor where the transesterification happens; then the products are transferred to a separator where the biodiesel is extracted and then washed with water to eliminate any residues. The glycerol and alcohol are also separated and recovered, the former is reused in the process and the latter is a co-product.

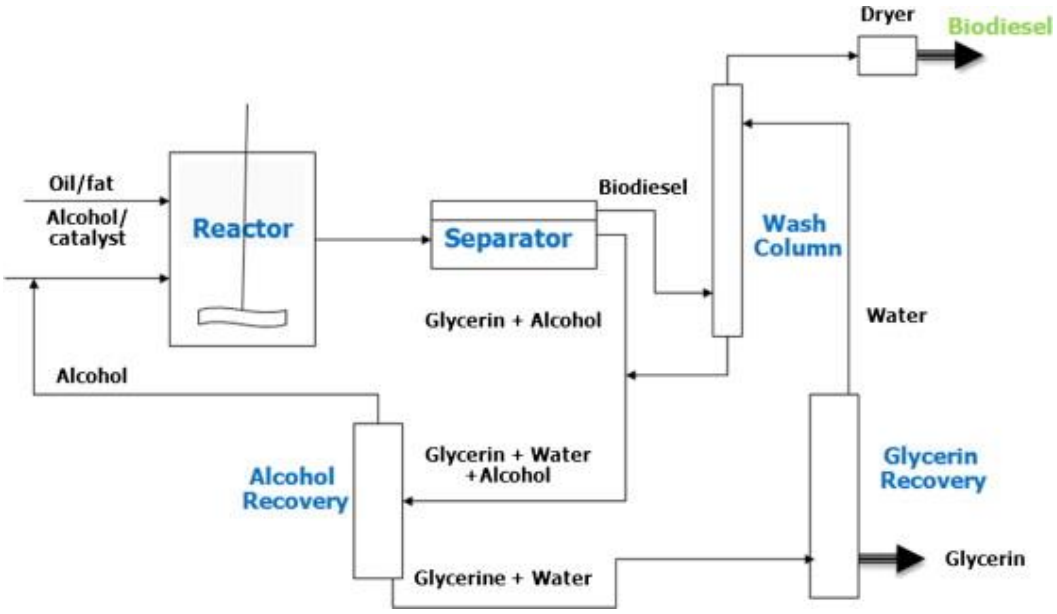

Figure 2.1: Industrial process of biodiesel production by catalytic transesterification (Abbaszaadeh et al., 2012)

Vegetable oil is mainly composed of fatty acid chains of 8-22 carbons (the main ones are presented in Table 2.1) which are stored as triacylglycerol (TG) in most feedstocks; some are also presented in the FFA form.

Table 2.1. Fatty acid list (Tyson et al., 2004)

Fatty Acid Name	Number of Carbons & Double Bonds	Chemical Structure
Caprylic	C8:0	$CH_3(CH_2)_6COOH$
Capric	C10:0	$CH_3(CH_2)_8COOH$
Lauric	C12:0	$CH_3(CH_2)_{10}COOH$
Myristic	C14:0	$CH_3(CH_2)_{12}COOH$
Palmitatic	C16:0	$CH_3(CH_2)_{14}COOH$
Palmitoleic	C16:1	$CH_3(CH_2)_5CH=CH(CH_2)_7COOH$
Stearic	C18:0	$CH_3(CH_2)_{16}COOH$
Oleic	C18:1	$CH_3(CH_2)_7CH=CH(CH_2)_7COOH$
Linoleic	C18:2	$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$
Linolenic	C18:3	$CH_3(CH_2)_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$
Arachidic	C20:0	$CH_3(CH_2)_{18}COOH$
Eicosenoic	C20:1	$CH_3(CH_2)_7CH=CH(CH_2)_9COOH$
Behenic	C22:0	$CH_3(CH_2)_{20}COOH$
Eurcic	C22:1	$CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$

The transesterification reaction happens in three stages, as exemplified in Figure 2.2, where the TG and FFA reacts with an alcohol that has low molecular weight (usually methanol or ethanol), usually in a presence of a base or acid catalyst. The reaction also produces glycerol as a co-product. The stoichiometry requires 3 moles of alcohol to each mole of triglyceride, but for a higher yield, since the reaction is reversible, this ratio needs to be much larger, such as 30:1 (Lotero et al., 2005).

Methanol is the most used alcohol because of its low cost and easy accessibility, but ethanol has been studied as its replacement because it can be produced from sugars by a renewable route (Moser, 2009). The problem with ethanol is that it produces smaller yields and the glycerol separation is harder (Meher et al., 2006). The catalyst can be alkaline, acidic, or enzymatic with sodium

hydroxide (NaOH), potassium hydroxide (KOH) and sulphuric acid being the most commonly used.

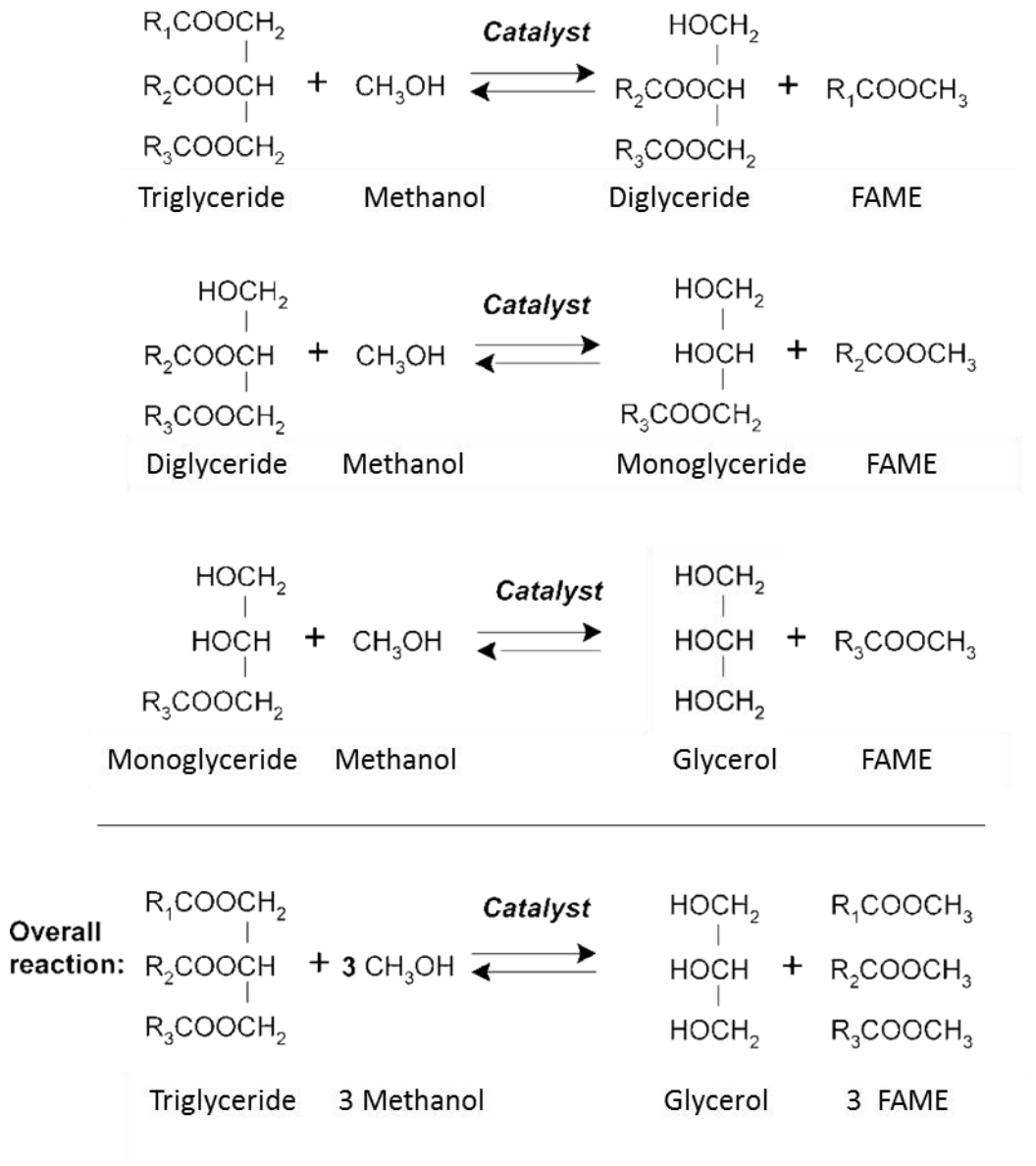

Figure 2.2. Transesterification of triglyceride reaction with methanol (Lotero et al., 2005)

Vegetable oils usually contain water, FFA, phospholipids, sterols and other impurities that have significant impact in the catalyst transesterification process. The water reacts with monoglycerides and forms FFA which together with the catalyst generates soap instead of the desire product. The soap presence

decreases the yield, consumes the catalyst and makes the separation between biodiesel and glycerol harder (Ma and Hanna, 1999).

2.4. Biodiesel generations

Oil for biodiesel production is usually from soybean, rapeseed and other crops. Depending on its source, the fuel has been categorised into three generations. Crops already used for food, such as soy and palm represent the first generation, the new non-food crops cultivated for fuel production such as castor beans and *Jatropha curcas* form the second generation, and algae forms the third generation (Abdelaziz et al., 2013a).

The biofuels need to decrease their cost to make their products economically feasible, especially when competition for resources, such as land and water, with other markets crops are taken into consideration (Ben Fradj et al., 2016). Moreover, some resources indicates that deforestation has been caused by large scale crops of first generation feedstock (Gumba et al., 2016). Therefore third generation biofuels could be a viable option to produce fuel as it does not require arable or productive areas (Demirbas and Fatih Demirbas, 2011), but more studies are necessary to measure the footprint and fertilizers requirements for the algae cultivation.

A summary of the three generations with their main feedstocks , benefits and problems is presented by Gumba et al. (2016) and shown in Figure 2.3.

Figure 2.3. Biodiesel generations based on feedstock (Gumba et al., 2016)

2.5. Algae as biomass

Research to obtain fuel from algae is not new. It was initially proposed in 1950 (Rodolfi et al., 2009), and with the oil crisis in the 1970s several public funding research programs started, but when the crude oil price fell (around the 80s) the programme of research was halted (Campbell et al., 2011). Today this topic is again at the forefront, with governments and companies investing in R&D, with a variety of algae species being tested, technologies of cultivation and oil extraction have been improved and pilot plants have been constructed.

Some examples of these investments can be seen by the Bioenergy Technologies Office of U.S. Department of Energy (US), the Algal Bioenergy Special Interest Group of Natural Environment Research Council (UK) and Ministry of Science and Technology – MCT (Brazil). There have also been private investments from companies such as Algenol, BP, Shell, Chevron, ExxonMobil and others.

There are more than 30,000 species of algae described (Guiry, 2012). When classified by size algae are divided into:

- Macroalgae (‘‘seaweeds”):
    - Macroalgae are multicellular plants (large algae, visible without a microscope) growing in salt or fresh water. They can be brown seaweed (*Phaeophyceae*); red seaweed (*Rhodophyceae*) and green seaweed (*Chlorophyceae*) based on their pigmentation (Demirbas, 2010).
- Microalgae:
    - Microalgae are unicellular photosynthetic micro-organisms and they can be defined as eukaryotic organisms that contain chlorophyll A and a plastid. This excludes cyanobacteria but several biofuels’ studies include this group because of their properties and potential (Abdelaziz et al., 2013a).

Microalgae can grow autotrophically (when supplied with light, CO_2 and nutrients), mixotrophically or even heterotrophically (using organic substrates such as sugars). Heterotrophic growth may also enhance lipid production (Abdelaziz et al., 2013a).

Algae are cultivated in aquatic media (seawater, fresh water, brackish water and domestic and industrial effluents) using nutrients and CO_2 as inputs. The nutrients, mostly nitrogen and phosphorus, can be provided from fertilizers

or wastewater while the CO_2 is usually provided by a pump system from power plants or industries that emit this gas. 1 kg algae biodiesel would require 3726 kg water, 0.33 kg nitrogen and 0.71 kg phosphate without recycling the wastewater and less 84% in the water usage and 55% in nutrients with recycling (Yang et al., 2011), so there is the necessity of recycling since the use of fertilizers would return to the problems of the first and second generation of resources requirements. Researchers conclude that the only cost-effective strategy to produce microalgae on a large scale would be to construct the facility close to a source of nutrients and CO_2 (Abdelaziz et al., 2013a).

Algae can grow on non-arable land in different environments (even under harsh conditions), so there are lower or no negative impacts in land-use change (Abdelaziz et al., 2013a).

The advantages of using algae as feedstock includes:

- Non-food based feedstock resources;
- Non-productive and non-arable land can be used for growth;
- High density per area productivity;
- High quantity of lipids (can achieve more than 40% wt. of their dry biomass, compared to 25% of rapeseed).
- High photosynthesis capability (10% compared to 1% of regular crops);
- Fast growth cycle - few days;
- Valuable co-products can be recovered during biofuel production (Parmar et al., 2011);

- Capability of recycling waste CO_2 emissions (Gallagher, 2011); 100 tons of microalgae biomass fixes 183 tons of CO_2 (Chisti, 2007);
- Applications of pesticides, herbicides or fungicides are not necessary (Costa and de Morais, 2011);
- Biofuels are more stable due to the greater carbon and hydrogen content (1.72 H/C molar ratio compared with 1.38 of plant biofuel) and lower oxygen content (0.26 O/C molar ratio compared with 0.37) (Costa and de Morais, 2011).

Comparatively, the novel technology also brings some challenges. The cultivation design is not developed for large scale and ponds have problems with contamination and the evaporation of water, whilst bioreactors have problems keeping the light distribution and temperature in the system. The high nutrient requirement (nitrogen and phosphorus) can impact in agricultural market as algae demand nutrients that were before only allocated to other crops. According to Demirbas (2010), the need for fertilizers can be 111 times greater than that required by rapeseed production, so there are the need for recycling it from wastewater for example. Another challenge faced in the use of algae as feedstock is the market for higher value algal products such as proteins and omega-3, so the biorefinery approach needs to be considered (Brentner et al., 2011). At this moment the cost for algae cultivation is greater than the value of the fuels that can be produced by it and the technology needs to be developed in order to make the large scale cultivation feasible (Abdelaziz et al., 2013a).

Algae oil is composed mainly of saturated or unsaturated fatty acids from 12 to 22 carbons in length; predominantly C16:0, C18:1, and C18:2, with a

favourable profile for fuel production (Halim et al., 2012). After the lipid extraction, the remaining biomass can be used to produce other biofuels, such as jet fuel, biogas or ethanol, and can produce useful by-products including animal feed, anti-oxidants, colouring substances, fertilizers and soil conditioners, cosmetics, pharmaceuticals and others (Parmar et al., 2011).

2.6. Biodiesel from algae

The process to produce biodiesel from algae can be divided into six main phases after algae selection and are presented below:

- Algae selection:
  - Strain;
  - Cultivation conditions;
- Cultivation – growing the algae:
  - Cultivation method – open pond, photobioreactor or fermenters;
  - Control factors – climate (temperature and solar irradiation), CO_2, nutrients;
- Harvesting and dewatering – to obtain the biomass;
- Drying – feedstock processing;
- Oil extraction;
- Conversion – from algae oil to biodiesel;
- Oil refining.

2.6.1. Selecting the strain

The algae selection is considered the first step in the construction of the biodiesel facility. According to Abdelaziz et al. (2013a) selecting a single (or

several) strains of algae to produce biodiesel with current technologies on a large scale is not possible due to the low rate of success in long-term growth. The selection process should contemplate the following issues (John et al., 2011):

- High oil-biomass ratio, i.e. a high lipids content;
- Compatibility with the climate at the facility location:
    - Resistant to seasonal variations and diurnal cycle;
- Easy cultivation:
    - Predictable behaviour and maintenance of high oil content under stress;
    - Resistance against pests;
    - Nutrient requirements easily met;
- High CO_2 sink capacity;
- Valuable co-products.

The quantity of lipids is one of the most important characteristic of algal biodiesel production as it enables the calculation of how much oil will be produced. The lipid content in macroalgae is around 10% wt. (Gosch et al., 2012) and in microalgae varies between 20% to 80% wt. (amounts between 15% to 50% wt. are typically extracted) (Chisti, 2007).

2.6.2. Cultivation

The cultivation stage is where the algae grow with the consumption of energy, CO_2, water and nutrients. Usually it is the most expensive stage, representing more than 60% of the total cost (Ventura et al., 2013). It can be done in an aquatic farm using different approaches: sea, ponds, photobioreactors or fermenters (for heterotrophic microalgae).

The cultivation by open raceways pounds is considered the best industrial option, followed by photobioreactors (PBR) – tubular, flat-plate and column (Clarens et al., 2010). Nowadays ponds and photobioreactors are used on small commercial scales in the market (for the production of high value products such as proteins, pigments, animal feed, etc.) and because of this, information for those techniques are more readily available (Milledge, 2011).

Each cultivation method has advantages and disadvantages. While PBRs present better results of productivity and avoid the loss of water by evaporation; they are more expensive, more difficult to operate and use more energy than open ponds. In light of this, both technologies need to be improved (Norsker et al., 2011).

Some companies are also testing and creating new technologies. One example is the photobioreactor made of a polymer material that floats on the sea created by Algasol Renewables (2014).

2.6.3. Biomass processing (harvesting, dewatering and drying)

Harvesting is usually done by the addition of synthetic or biological flocculants, whilst a rotary press or filter press can be used to dewater the biomass (Lardon et al., 2009). This process can also be done by centrifugation or filtration. Examples of flocculants are aluminium sulphate (Stephenson et al., 2010), hydrophobic polymer (Campbell et al., 2011) and chitosan (Brentner et al., 2011). Chen et al. (2015) presented in their work a review of the available methods for dewatering and drying. Figure 2.4 shows the main routes they

proposed. After the dewatering (by flocculation, filtration or centrifugation) the algae are dried by freeze drying (used in lab scale), spray drying, convective drying or solar drying.

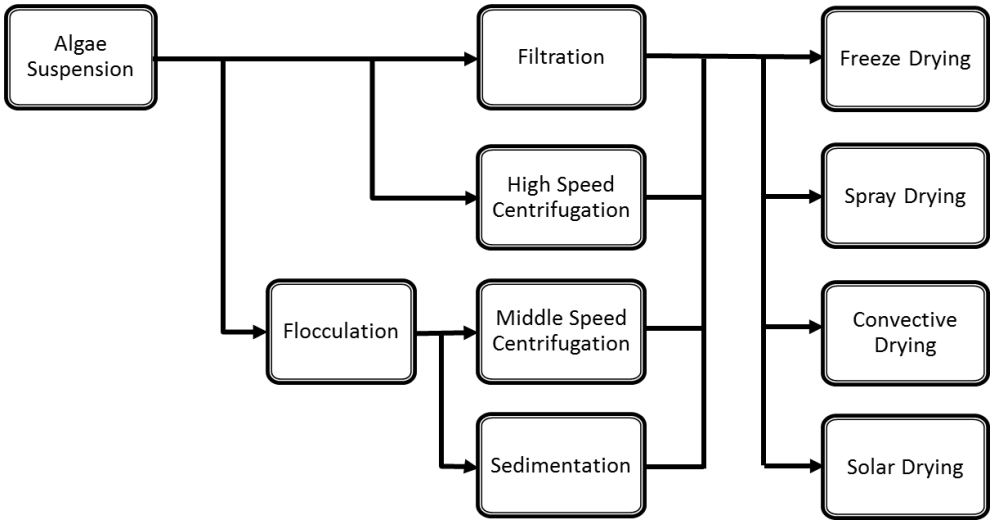

Figure 2.4. Dewatering and drying processes (Chen et al., 2015)

The literature suggests the solar drying or the convective drying are the only methods that could be applied in large scale for the biodiesel production (Chen et al., 2015). The solar drying is the less energy demand and cheapest method, but it requires a large surface space and it is time consuming. Therefore, biomass drying is a limiting process for the use of microalgae as feedstock for fuel production.

2.6.4. Oil extraction

The method most adopted to extract oil from the algae biomass is dry extraction with hexane as the solvent. Studies state that this method emits less GHG when compared to wet extraction and secretion (Vasudevan et al., 2012, Torres et al., 2013).

According to the literature, wet extraction is possible and eliminates the dryer process (Sills et al., 2012). Different methods are suggested in the literature, and the wet extraction can be done through centrifugation and wash cycles (Vasudevan et al., 2012) or hydrothermal liquefaction (Sills et al., 2012). These technologies need development, as there is not an industrial-scale wet process available (Lardon et al., 2009).

An alternative method of oil extraction is the use of supercritical fluids. Brentner et al. (2011) argue that this technology avoids the use of solvents, avoid the drying process and also, when methanol is used, can be combined to directly convert oil to diesel. Several researches have been conducted to find the best conditions for biodiesel production from biomass oil (Kusdiana and Saka, 2004, Vieitez et al., 2008, Silva and Oliveira, 2014). The concern about the use of supercritical fluids is the amount of energy used to achieve the high temperature and pressure (around 31°C and 73 bar with CO_2 and 240°C and 78.5 bar with methanol) and the cost of the required equipment.

2.6.5. Algal oil conversion to biodiesel and refining

The conversion process is assumed to be the same one currently used for other feedstocks; this is, usually, a basic transesterification with methanol, using sodium or potassium hydroxide. Sulphuric acid can also be used for acid transesterification (Chowdhury et al., 2012) and the conversion can be done by supercritical alcohol (Brentner et al., 2011).

The biodiesel is then separated from the remaining solvent, catalyst and co-product; and refined as necessary. The solvent and catalyst are redirected to be reused in the process while the glycerol is sent to the market.

This research studies the use of supercritical ethanol technologies for simultaneous extraction and transesterification of oil, thus eliminating the drying process. An impact and energy demand analysis is also considered to investigate its feasibility. This process has not been tried with wet algae in a continuous process.

2.7. Supercritical fluids

The use of supercritical alcohol has already been studied for the transesterification of other vegetable oils (Velez et al., 2012). A supercritical condition is considered when the temperature and pressure are above the critical point (critical temperature and pressure of the fluid/solvent). These conditions are able to decrease the reaction time by reducing mass-transfer limitations, as the polar phase (alcohol) mixes with the non-polar phase (oil) on a single homogenous phase (Vyas et al., 2010). The solvent and transport properties of supercritical fluids are better than in gas or liquid states, with a low viscosity, high diffusivity and low surface tension, so its mass transfer rates are enhanced (Gumba et al., 2016).

Under supercritical conditions, the alcohol has its solvent power increased and its polarity modified (Bazaev et al., 2007) which would allow it to act as a

catalyst and reagent simultaneously in the transesterification process and eliminates the use of acids or alkyls in the reaction.

2.7.1. The new route – Supercritical ethanol to produce biodiesel

Supercritical ethanol has been used in the direct extraction and transesterification of biomasses lipids, such as rapeseed and soybean oil (Silva et al., 2014, Reddy et al., 2014). This approach eliminates the catalyst use, therefore removing its separation and filtration procedures.

The biodiesel yield can be influenced by the parameters of supercritical transesterification. Those typically are the reaction temperature, pressure and time, alcohol reagent used, its proportion to oil (molar mass), extra solvents and catalyst presence (Wen et al., 2009).

Increasing the reaction temperature has shown to be favourable to the yield until the temperature where the alcohol starts to decompose the FAME or FAEE, which occurs around 350°C (Olivares-Carrillo and Quesada-Medina, 2011).

The reaction time in supercritical conditions is much shorter than a catalytic process. The reaction can be completed in less than 10 minutes while in the presence of a catalyst this can usually be up to a couple of hours depending on the contacting/mixing device (Wen et al., 2009).

Water in catalytic transesterification inhibits the reaction (Laurens et al., 2012, Ghasemi Naghdi et al., 2014). The monoglyceride reacts with the water and forms FFA instead of FAEE (Figure 2.5). Sulfuric acid could achieve only 10% of the

yield potential when the water amount is increased above 6 M, with methanol:acetic acid 2:1 M and 10^{-3} M of catalyst at 60°C (Liu et al., 2006). The opposite happens with the addition of water to the supercritical environment. Water has been shown to improve the hydrolysis of the triglycerides and 50% vol. of water addition did not affect FAME yield at 350°C for 4 min (Kusdiana and Saka, 2004).

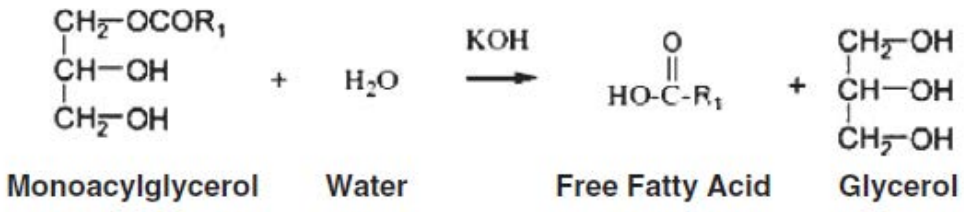

Figure 2.5. Water reaction in catalyst transesterification (Halim et al., 2012)

Another factor that is adverse for the catalyst reaction is the presence of free fatty acids that react with the catalyst and form soap. This consumes the catalyst and oil (Figure 2.6), increases the biodiesel viscosity and makes the glycerol separation more difficult (Demirbas, 2005). Under supercritical conditions the free fatty acids are esterified increasing the biodiesel yield.

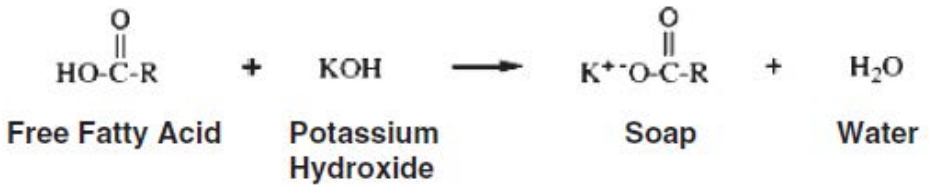

Figure 2.6. FFA reaction in catalytic transesterification (Halim et al., 2012)

The choice of alcohol also leads to different reactions with the oil. Warabi et al. (2004) studied the reaction with methanol, ethanol, propanol, butanol and

octanol at 300°C and 350°C with pressure from 60 to 430 bar depending of the alcohol. Using methanol at 300°C and 200 bar, 100% of the fatty acid alkyl esters resulted in 15 minutes while ethanol needed a retention time of 45 minutes (at 150 bar) and the other alcohols did not achieve the full conversion after this time.

The molar ratio between alcohol and oil also influences the yield produced. This ratio can change depending on the oil composition and can achieve values greater than 30:1 (Lotero et al., 2005).

Ethanol was selected in this work because it can be produced by renewable sources and consequently makes the process more sustainable. The yield produced by ethanol is usually smaller than the reaction with methanol, but when fuel properties are considered (such as oxidation stability, cetane number and cold flow properties) FAEEs are better than FAMEs (Reddy et al., 2014).

Ethanol reaches supercritical conditions at 240.9°C and a pressure of 63 bar. At this point ethanol has a density of 273 $kg \cdot m^{-3}$, compared to 789 $kg \cdot m^{-3}$ at standards conditions (Bazaev et al., 2007). Figure 2.7 shows the phase diagram of ethanol with the triple point and critical point represented.

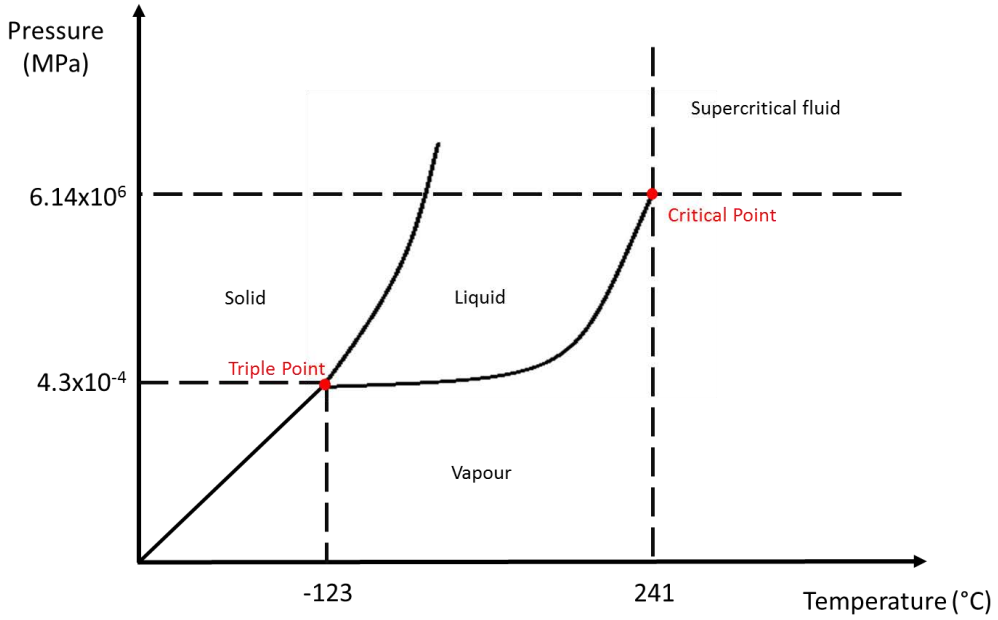

Figure 2.7. Ethanol phase diagram (axis not in scale)

According to Gui et al. (2009), the optimum conditions for oil transesterification in ethanol is at 349°C (pressure above 63.8 bar) and 30 min of retention time with a ratio of 33:1 ethanol:oil to give a yield of 79% wt.

The addition of co-solvents, such as CO_2, hexane or propane, has been studied to decrease the harsh conditions necessary to achieve supercritical conditions and also resulted in higher yields of biodiesel. Vyas et al. (2010) compared the transesterification of diverse vegetable oils (including sunflower oil, rapeseed oil, palm oil, etc.), and shows that conversion greater than 90% in less than one hour can be achieved.

Other methods to decrease the temperature and pressure in the supercritical fluid process can be achieved by the addition of a catalyst. Alkali, acid and enzyme catalysts have been tested, and have been shown to increase the

reaction rate, however there was no analysis of its separation afterwards to understand whether its addition is beneficial (Wen et al., 2009).

Most of the research at supercritical conditions has been made in a batch reactor (Reddy et al., 2014) but some laboratory work has been conducted in a flow reactor with retention time varying from 16.8 min to 52.5 min, 1:40 oil:ethanol molar ratio and up to 10% wt. water, at 350°C and 200 bar (Vieitez et al., 2008). The results from a flow reactor confirmed what was found in the batch process: increasing temperature, pressure and molar ratio enhances the biodiesel production yield and the reaction.

The flow reactor has the advantage of intensifying the heat transfer due to a larger area to volume ratio (Hartman et al., 2011). It is also safer and easier to operate than the batch reactor, with a better control of the parameters such as pressure. It makes the process faster because it can be integrated with downstream processes and it is not necessary to heat and cool the system every time (Harvey et al., 2003). The disadvantages include the price of equipment, such as pumps and connectors for the elevated conditions. The flow reactor is easier to scale up or –out with a continuous process.

As mentioned previously supercritical fluids have been studied for the conversion of vegetable oil, but there are few works that approach the direct conversion of algae biomass to biodiesel. The initial research was conducted using methanol as the solvent in a batch reaction with rehydrated *Nannochloropsis sp.* algae. The best conditions were found to be a ratio of 1:9 wt. wet algae to vol.

methanol, 30 minutes of reaction at 255°C and 82 bar (Patil et al., 2012). When ethanol was used, the maximum yield achieved was 67% at 265°C (no pressure reported) after 20 minutes of reaction with a ratio of 1:9 wt. dry algae to vol. ethanol (Reddy et al., 2014). The use of ethanol was also tested in *Chlorella sp.* with the addition of different catalysts. At 350°C after 60 minutes with a ratio of 5:24 wt. algae to vol. ethanol with 5% wt. $ZnCl_2$ the FAEE yield was 64.4% (Jin et al., 2014).

Throughout the literature there is no work on the direct transesterification of wet (un-dried) algae in a flow reactor. This approach negated the water amount in the biomass, eliminated the drying and oil extraction processes which would reduce the energy demand in the overall process. A complete energy and environmental analysis of this route for third generation biodiesel production was also not found in the literature.

2.8. Life cycle assessment and energy balance

A Life Cycle Assessment (LCA) is a method to evaluate the potential environmental impact (such as meaningful GHG emissions), including all inputs and outputs of the product system throughout its life cycle (ISO, 2006). In the biofuel studies, the life cycle should start with raw material production and ending with fuel consumption ("cradle-to-grave").

This approach is able to incorporate impacts that happen during the entire life cycle of a product and not just during its production. The scientific community

agrees that LCA is one of the best methodologies developed to assess the environmental loads associated with biofuel production (Requena et al., 2011).

LCA was launched in 1960 to analyse packaging alternatives and other bulk commodities (Council, 2004) and since then this technique have been improved and applied across numerous industries. The International Organization for Standardization (ISO) created standards which were reformulated and compressed in two documents: ISO 14040 and ISO 14044 (2006). The ISO does not display a single method to conduct LCA, but sets out that LCA should be done in four steps: goal and scope definition; life cycle Inventory (LCI); life cycle Impact assessment (LCIA); and interpretation. Figure 2.8 presents how they relate to each other. A brief explanation of each phase is given below.

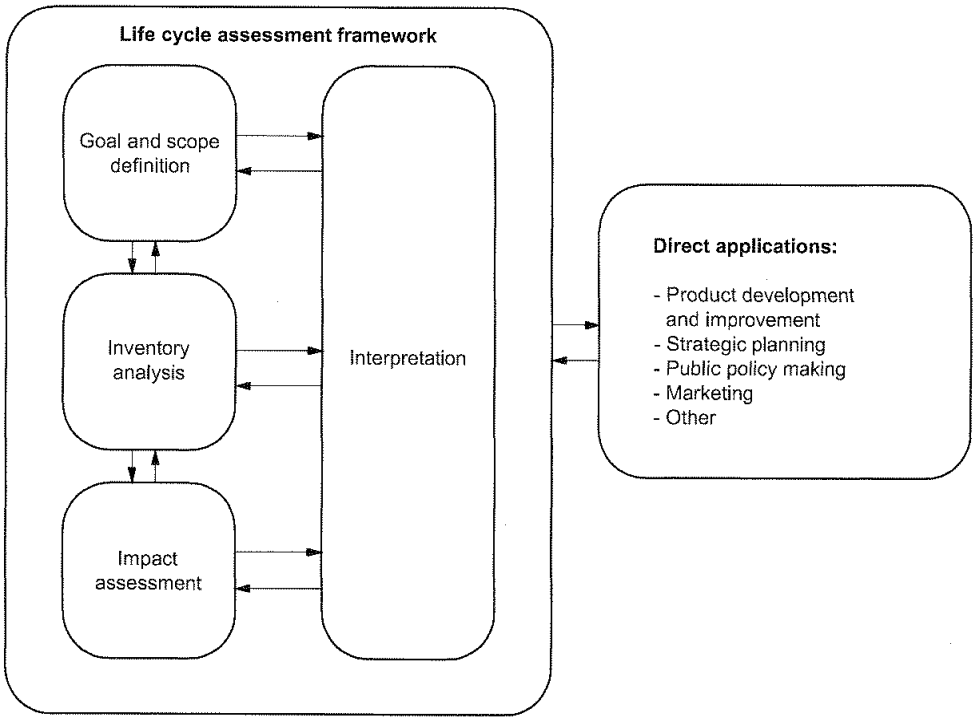

Figure 2.8. Stages of LCA (ISO, 2006)

2.8.1. Goal and scope definition

Defining the goal and scope of the study is to understand the objective of the study. This is where the intended application, audience, the reasons for the study, system boundary, data requirements, functional unit, assumptions, limitations and any other need for critical review are defined.

The goal could be an analysis of a system in order to understand the stages of the life cycle and identify what should receive more attention or change, or a comparative analysis to define which system is better when compared with others.

The functional unit is the unit which the study will be considered, and it is the basis for comparison. In the case studied, the functional unit will be 1 litre of biodiesel produced.

The system boundary is important to identify what is measured as the impact of this product and what occurs from outside the system, what delimitations of the inputs and outputs will be considered and whether it is necessary to allocate procedures.

The approach of LCA is then decided and it can be attributional or consequential (Kendall and Yuan, 2013):

- Attributional LCA – the system is evaluated through a static approach (inputs and outputs are tracked on a life cycle basis);

- Consequential LCA – changes from the environmental flows to the processes are considered to evaluate the system (e.g. land use change).

In this phase, some requirements for the next steps of the LCA are defined. It includes the methodology used, the data that will be collected, the way they will be collected (including the accuracy of collection to make the system study consist) and how the results will be presented.

2.8.2. Inventory analysis

During the inventory analysis, the flow diagram of the processes and the data collection plan are developed. Data are collected and calculation is then done to quantify inputs and outputs of the system; the information is analysed and organized and the results are evaluated and reported.

The data are divided in three categories: physical inputs, products and emissions; and its nature and accuracy depend upon each project. It can be measured, calculated or estimated, but all should be validated and the sources justified.

When there is more than one product being created by the system (co-products), the allocation procedure is adopted to quantify how the inputs and outputs should be divided. Allocation should be avoided wherever possible using devices such as dividing the process in to sub-process or expanding the system border to include the process related to the co-product (avoiding the multi-functionality). When the allocation cannot be avoided a relationship should be

established to attribute the data between co-products, this relation can be of mass, energy or cost.

2.8.3. Impact assessment

The LCIA is the evaluation of the results (potential human health and environmental impacts – natural environment and resources) obtained in the previous phase. It aims to associate inventory data with environmental impacts. In this phase, the goal and scope are reviewed.

The first stage is to select the impact categories, category indicators and characterization. They should be internationally accepted, scientifically and technically valid and environmentally relevant. One example is global warming (impact category) which is measured by the quantity of greenhouse gases emissions, such as carbon dioxide, methane, and others (indicators) and this is characterized as global warming potential in CO_2 equivalents (characterisation factor).

Some impact categories at the midpoint and endpoint level are presented in Figure 2.9. LCI results are assigned to the selected impact categories and then the indicator results are calculated.

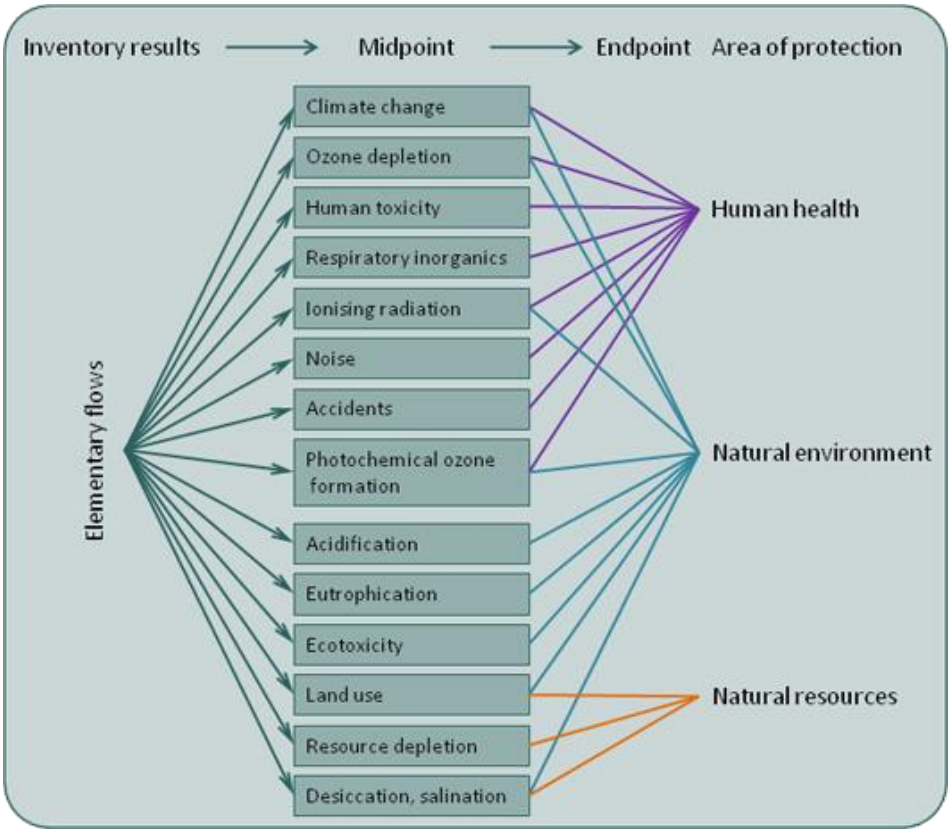

Figure 2.9. Framework illustrating some impact categories for characterisation modelling (E. Comission, 2013)

In additional, the author can apply normalization (calculating the magnitude of results relative to reference information), grouping (ranking the impact categories), weighting (aggregating indicator results using chosen numerical factors) and data quality analysis. These optional elements may change the results and they vary depending of the chosen source, and they can be viewed as a weakness of the process.

2.8.4. Interpretation

The interpretation phase consists in identifying, quantifying, checking and evaluating the results from the previous phases (LCI and LCIA), communicating them effectively according to the goal and scope defined at the beginning and

providing recommendations to the studied process. This is where the results, conclusion and limitations of LCA are presented.

After these stages, a report is compiled addressing the data, methods, assumptions and selections applied in the different phases of the study and before going through a critical review process.

2.8.5. Methodologies, tolls and databases

The ISO standards were built on international agreements, but they are not very detailed or prescriptive. It is therefore possible to find LCAs with different results by using the same framework given by the standards.

Some guidance documents and handbooks have been created because of this variability and uncertainty. The European Commission developed the International Reference Life Cycle Data System (ILCD) to increase the availability of coherent and quality-assured data, methods and LCAs. It is compatible with ISO standards and contains information from different sources resumed in a database called European reference Life Cycle Database (ELCD) (Comission, 2013).

Some researchers have also proposed new methods to undertake the LCA. According to Acquaye et al. (2012) there are three methods: the first one is analysing the process, such as defined by ISO, the second is called “Environmental Input-Output” (EIO) method, that uses country and/or regional input–output data coupled with averaged sectorial emissions to calculate environmental impacts,

and the last one is the hybrid LCA that couples both together. Currently, the hybrid model is more commonly used and accepted by the academic community.

Even with all this effort for standardization and guidance, it is hard to compare the studies that have been undertaken using LCA to analyse different biofuels. Kendall and Yuan (2013) deliberated this variability during the assessment and their conclusion is presented in the Figure 2.10.

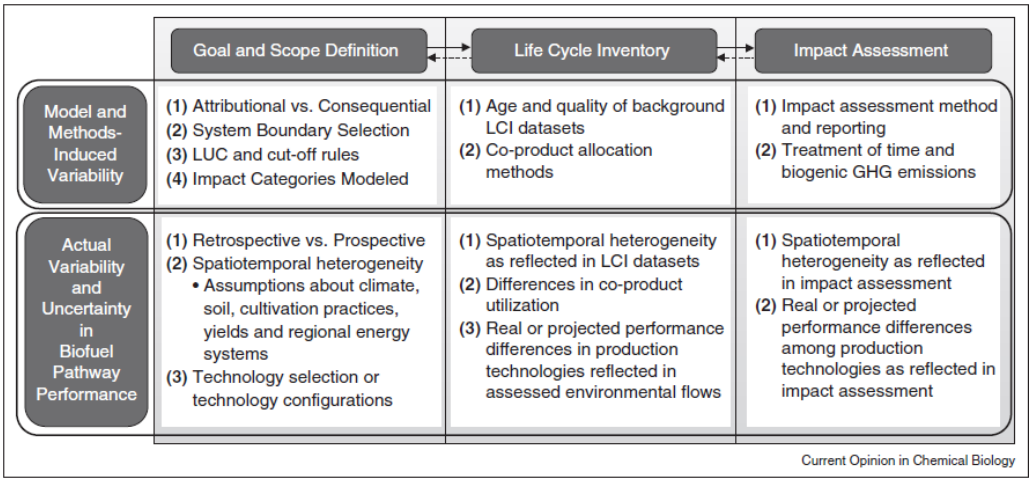

Figure 2.10. Sources of variability and uncertainty at each stage of a biofuel LCA (Kendall and Yuan, 2013)

LCA software has been developed to help the assessment. Some examples are GaBi, SimaPro, Quantis Suite, EarthSmart, and others. They are very similar, differing by the interfaces and the database they accept. The GaBi 6.0 software was chosen in this study due its availability and the recognition of the database used.

2.8.6. LCA previous studies – Algae biodiesel

Previous life cycle assessments of algae biodiesel were analysed in order to identify the main factors about the processes used and the viability of this kind

of fuel. In addition, the methods, technologies and impacts were identified. A table summarising this analysis is presented in Appendix 1.

Each author undertakes a LCA with a different goal and because of that they use different approaches and present the results in different ways. It is important therefore to identify the convergences and divergences between the studies and the results depending on each assumption. This kind of analysis is recent with the first significant study dated 2009 (Lardon et al., 2009).

The first issue about algae biodiesel LCA is the absence of commercial scale production. Currently, most LCA use lab-scale or pilot plant data with estimations to scale-up (Lardon et al., 2009) or use data from previous studies (Yang et al., 2011). The scale-up process still faces criticisms, as factors could be changed in an industrial scale. One example is presented by Passell et al. (2013) who collected data from commercial algae producers (1,000 m^2 – more than 500 times smaller than a real biodiesel facility should be) and stated that the productivity reported is much smaller than currently assumed (3 $g \cdot m^{-2} \cdot day^{-1}$ against 30 $g \cdot m^{-2} \cdot day^{-1}$).

In light of the feedstock chosen, it is possible to see that a large number of the studies do not specify the microalgae species used and in some studies the species is just referred to by the productivity (L $oil \cdot ha^{-1} \cdot year^{-1}$) and not at all by the process. *Chlorella vulgaris* and *Nannochoropsis sp.* are the most used cultures (Passell et al., 2013, Jorquera et al., 2010, Lardon et al., 2009), because of their high lipid accumulation, easier cultivation and high environment adaptability

when compared to other species. About 70% of LCA studies use chemical fertilizers even though wastewater would be more viable in a commercial scale (Lam and Lee, 2012). Other species are also mentioned, such as *Botryococcus braunii*, *Dunaliella tertiolecta*, *Haematococcus pluvaris*, etc. (Campbell et al., 2011).

The selection of microalgae species depends on various factors such as oil (lipids) content, compatibility with the climate at the facility location (resistance to seasonal variations and diurnal cycle), easy cultivation (resistance against pests, predictable behaviour under stress, etc.), high CO_2 sinking capacity, low energy requirements to oil extraction (such as thin wall cell), valuable co-products, and others (John et al., 2011). The choice can have consequences to the LCA and it is necessary to measure these impacts with a specific LCA for the selected feedstock.

Another factor to consider is the facility location. Most studies took place in the USA (or using data from this country) (Brentner et al., 2011, Clarens et al., 2010, Passell et al., 2013) or in the Mediterranean area, such as France and Spain (Torres et al., 2013, Delrue et al., 2012). Some studies have been undertaken in Australia (Campbell et al., 2011), Singapore (Khoo et al., 2011), Brazil (Galindro, 2012) and Korea (Ventura et al., 2013).

Shirvani et al. (2011) made a comparison between UK, France, Brazil, China, Nigeria and Saudi Arabia considering different input energy grids, but the facility location also changed other factors, such as weather conditions (this

influences the production of oil by hectare of cultivated algae), available land and accessibility of resources (nutrients, energy and other inputs), it is important to make a more detailed study in each location in order to compare differences between them.

The location influence was also presented in an education tool (Algal Raceway Simulator) created by the Massey University, University of Queensland and Algsim Limited, where the user can simulate the productivity, water demand and temperature in a raceway pond, choosing the location, pound depth, retention time and recycling ratio (AlgApp, 2013).

The boundary also diverges between the studies. The most complete LCA scenario, suggested by ISO 14040 (2006), would be “well-to-wheel” or, as it is more commonly known, “cradle-to-grave” (Sills et al., 2012, Passell et al., 2013, Lardon et al., 2009), however the largest analysis is made “well-to-gate” or “well-to-pump” (Sander and Murthy, 2010, Torres et al., 2013, Delrue et al., 2012, Borkowski et al., 2012). This analysis excludes the biodiesel combustion (final use) which makes this scenario not comparable with other fuels, however it is often better to compare technologies and processes of the same fuel because it can reduce the imprecisions associated with the efficiency of the motor and the blending used.

Other problems related with the boundaries of the studies are related to the process inputs and production of co-products. The discussion encompasses the sources of the goods and whether they should be included inside the

boundary or whether they should be treated as inputs/outputs; and these assumptions can change the entire LCA.

The variations in the inputs start during algae cultivation where CO_2 and nutrients need to be provided. The best option of CO_2 source is power plants, because they are the biggest CO_2 emitter; other CO_2 options include delivery directly as flue gas from an ammonia plant or by truck in liquefied form (Campbell et al., 2011).

Most of the studies use synthetic fertilizers as nutrient sources, such as nitrogen as calcium nitrate or urea and phosphorus as superphosphate. These sources of nutrients are easier to apply in lab scale (to control the content provided), but it is agreed that the feasibility of the production would be improved with the use of wastewater that contains nitrogen and phosphorus. In a similar manner, there is one study that assumed shrimp effluent as source of the nutrients (Galindro, 2012) and others sources could be considered.

Environmental impacts can also be decreased if the methanol input during the transesterification was replaced by ethanol. However, this needs more study and currently is not used in the market because of the final characteristics of the biodiesel produced.

Allocation is usually done in the oil extraction process when crude oil and oil cake are generated. Later there is also allocation between biodiesel and glycerol. The creation of valuable co-products from the biomass cake and glycerol

is essential to ensure the feasibility of biodiesel production. The allocation is done by mass, energy or price. Mass and energy factors appear to be more precise ways to assess the environmental impacts, but the main factor in the market is the biofuel price. The cost associated to biodiesel production could decrease with a market for its co-products. The algae biomass can be allocated by expansion to produce a lot of products such as ethanol (Sander and Murthy, 2010) or biogas in an anaerobic digester (Campbell et al., 2011, Frank, 2011) whilst the glycerol can be directed to the pharmaceutical, food and cosmetic industries. Because of this, it is unfeasible to include these processes in the same LCA.

The disagreement to select the functional unit is even greater; the variations include unit of mass or energy content in the final biofuel or in the biomass. This choice increases the difficultly in comparing the LCA studies, since there is no consensus on the mass density and energy content of algae oil and biodiesel (Collet et al., 2014b).

The presentation of the results varies according to the goal of the project. Most of the studies calculate, as expected, indicators of energy (such as energy demand and net energy ratio) and air pollution (such as GHG emissions and global warning potential). These measures reflect the larger concerns about fuel use, whether it is energetically feasible and if it is better for the environment.

Other analyses are water use, land use (done usually by authors whose goal is to compare between feedstocks), cost of production (Gallagher, 2011,

Davis et al., 2011) and also other environmental and human impacts such as eutrophication and acidification.

A way to make the analysis easier and amalgamate the measures is by normalization methods. One example is the use of Eco-indicator 99 used by Torres et al. (2013) which calculates one unique value to represent the total environmental impact. Problems with this kind of technique result from the loss of details in the results and the increase in standard deviation.

The process analysed varies within and between the LCA studies. Authors agree that algae biodiesel production can be divided into at least four distinct processes: cultivation, harvesting and dewatering, oil extraction and conversion to biodiesel with four hotspots of energy requirement (nutrients source, photobioreactor operation (cultivation method), dewatering / biomass drying and lipid extraction (Lam and Lee, 2012)).

The transport distance of the oil to the biodiesel plant is considered by some authors and it varies from 0 km (cultivation and conversion in the same place) to 150 km (Sander and Murthy, 2010) or even 650 km by ship (Stephenson et al., 2010). The transport to the pumps is also considered in most studies as the same as biodiesel from other feedstock (the use of around 3.78 MJ for each kg of biodiesel) (Batan et al., 2010).

According to various studies, algae biodiesel is technically feasible (Chisti, 2007), but it still not feasible financially and even sometimes environmentally. In

2013, ExxonMobil announced that they would need more than 25 years to achieve the economic viability (after investing more than US$ 600 million in algae genetic technology since 2009 (Carroll, 2013)). On the other hand, Algenol (a small risk company) announced algae commercial production in 2014 to produce ethanol (33.750 litres in 0,4 ha) (Mota and Monteiro, 2013).

A common concern is the uncertainty associated in the assumptions during the LCA. Since each article assumes one technology and method to calculate the LCA, it is difficult to compare raw values between them. Sills et al. (2012) conducted this analysis and concluded that LCA should be used to report ranges of expected values to help decisions makers and not to conclude the viability of the biofuel. This was also stated by Razon and Tan (2011) who defined that the LCA can be used as a diagnostic tool to detect improvement options.

Considering the costs of a large scale system, a specific case – autotrophic algae growing in an open pond and a photobioreactor with harvesting by auto-flocculation, flocculation with chitosan followed by a centrifugation and solvent extraction with butanol to produce hydrotreated diesel – still did not compete with fossil-derived diesel (Davis et al., 2011). It is important to emphasize that it was chosen as a process technology that has been used in industry and not the best available, and the authors state that there is potential to reduce costs by technological and biological improvements.

On the other hand Gallagher (2011) concludes that the algae process is economically viable (considering the future price of oil) with government

subsidies in the beginning of its commercialization. He calculated the cost based on a 1,000 acres illustrative project with open ponds and stated that the largest expenses are the cost of feedstock (70%) followed by the operation (including sales and administration, alcohol, utilities, maintenance, insurance, labour, benefits and catalysts) (23%), and the final percentage as losses by depreciation.

Even with significant studies about the costs of the process, there are diverging opinions about financial feasibility.

Algae biodiesel is more “environmental friendly” than petrol-based diesel, but it remains questionable when compared to other biodiesels. According to Batan et al. (2010) algae biodiesel is better than soybean biodiesel, considering the net energy ratio and GHG emissions. The results can however be positive or negative depending on the chosen process (Clarens et al., 2010) and can be directly affected by parameters of energy and nutrient recovery (Frank, 2011); as a result more LCA are necessary.

One reason for the above observation is the premature technology that needs more development to decrease the energy use and the fossil fuels inputs. Solutions include the use of wastewater to provide nutrients and also the development of less energetic techniques during the process.

Most of the studies agree that energy consumption is the biggest challenge to the feasibility of the microalgae biodiesel production. By reducing the energy demand, it is possible to directly reduce some environmental impacts,

such as global warming potential, acidification, etc. (Lardon et al., 2009). One critical issue to future research is how to decrease the energy consumption during the oil extraction (the main consumer of energy, reaching up to 85% (Khoo et al., 2011)). The source of energy used in the drying process can also significantly change the energy demand impacts (Vasudevan et al., 2012).

This energy demand impact is also influenced by the source used during the process related to the location of the facility. Countries like Brazil and France (where non-fossilized electricity grids are used) have shown big potential for the development of algae biodiesel as a renewable alternative (Shirvani et al., 2011).

Even with the high energy demand, algae biodiesel can be considered a by-product in sewage treatment or in Carbon Capture and Storage (CCS) plants (Razon and Tan, 2011).

One way to decrease the energy demand, is the integration with anaerobic digestion and nutrients recycling (Chowdhury et al., 2012) which would emphasize the significance of inputs/outputs; other possible solutions are the elimination of the drying process as proposed in this thesis.

3. Potential of Third Generation Biodiesel

3.1. Introduction

In order to incentivise the use of biofuels a number of strategies have been created, such as mandatory targets and blending, and financial incentives (tax reduction, exemptions and subsidies). In addition, governments can intervene in the production chain by supporting some feedstock crops, subsidizing specific factors of production, granting incentives or creating import tariffs (Sorda et al., 2010).

This work evaluated the policies and governmental action focusing on three distinct places around the world (European Union (EU), United States (US) and Brazil). The area requirements to cultivate algae as a biodiesel feedstock in order to (i) meet the targets and (ii) replace current diesel products was analysed in the three cases. Possible locations to begin the cultivation of algae were identified.

The results presented in this chapter were published in the paper "Assessment of algae biodiesel viability based on the area requirement in the European Union, United States and Brazil" at Renewable Energy (Speranza et al., 2015).

3.2. Materials and methods

An analysis of policies from the world, EU, US and Brazil was completed based on official documents divulgated internationally in order to assess the requirements in biodiesel fuel demand and the role that the algae as feedstock could play in the biodiesel market.

The area requirement to produce biodiesel was assessed following two steps. The biodiesel requirements were calculated using data released by international governments in order to (i) achieve the established targets and (ii) replace the use of diesel projected to 2020. The area was obtained dividing this biodiesel demand by the algae biomass productivity based on seven scenarios established using assumptions from the literature for microalgae growth in open pond (OP), photobioreactors (PBR) and for macroalgae.

Following this, cities where algae might be produced nearby were identified using GIS software by considering the conditions necessary to cultivate algae. These included favourable temperature, area availability (uncultivated, unprotected and unoccupied areas) and proximity of necessary inputs, such as CO_2; other land use has been not considered. The data were obtained from the literature (ArcGis-Online, 2014, ArcGis-Online and Protectedplanet, 2014, GEO, 2014).

3.3. Results and discussion

3.3.1. Policies and targets

Driven by the growing debate over global warming and other environmental problems, governments and organizations started to create policies and targets in order to decrease GHG emissions and other pollutants. One way of achieving this and one of the biggest interests in research is the change in the energy matrix, which includes the creation of mandates and/or incentives to encourage the use of biofuels, such as biodiesel.

According to (REN21, 2016), in 2015 the global investment in the renewable capacity was US$286 billion and there were 173 countries with target policies and 66 with biofuels mandates.

Sometimes these strategies do not have the expected result. Sorda et al. (2010) showed in their work that biofuel production can be blamed for a rise in food prices in the US when, in 2006, 20% of the corn supply in the country was reallocated to ethanol production. The direct comparison between corn being used for food, and biofuel is not a fair comparison as there are many co-products which are produced in addition during the fuel manufacturing process which impact a variety of other markets (Tomei and Helliwell, 2016).

Since biofuel policies can configure changes and consequences in all markets, they should be based on energy, environment, agriculture and trade at the same time (Stattman et al., 2013). This means they must contain strong

interactions between energy policy, transport, agricultural market (food production) and forestry (Ward and Inderwildi, 2013). Policies in this field should therefore consider:

- Energy security and availability;
- Environmental impacts and land use change;
- Competition with agricultural products;
- Social configuration;
- Quality of the biofuels;
- Importation and exportation of feedstock and fuels.

To help assure the effectiveness of the policies, some sustainability certification could be created. One example is the “green stamp” in Brazil that aims to ensure the social sustainability of biodiesel (e.g. feedstock from family farms) (Brasil, 2012).

The European Commission summarizes this concept nominating in the 2006 Green Paper three fundamental principles: security (supply availability), competitiveness (price affordability) and sustainability (environmental dimension) (Afionis and Stringer, 2012).

The government interventions consist of mandatory targets for blending, financial incentives, such as tax reduction, exemptions and subsidies, and others. Besides that, governments can intervene in the production chain by supporting some feedstock crops, subsidising specific factors, granting incentives or creating import tariffs (Sorda et al., 2010). Figure 3.1 illustrates possible subsidies during the biofuel supply chain.

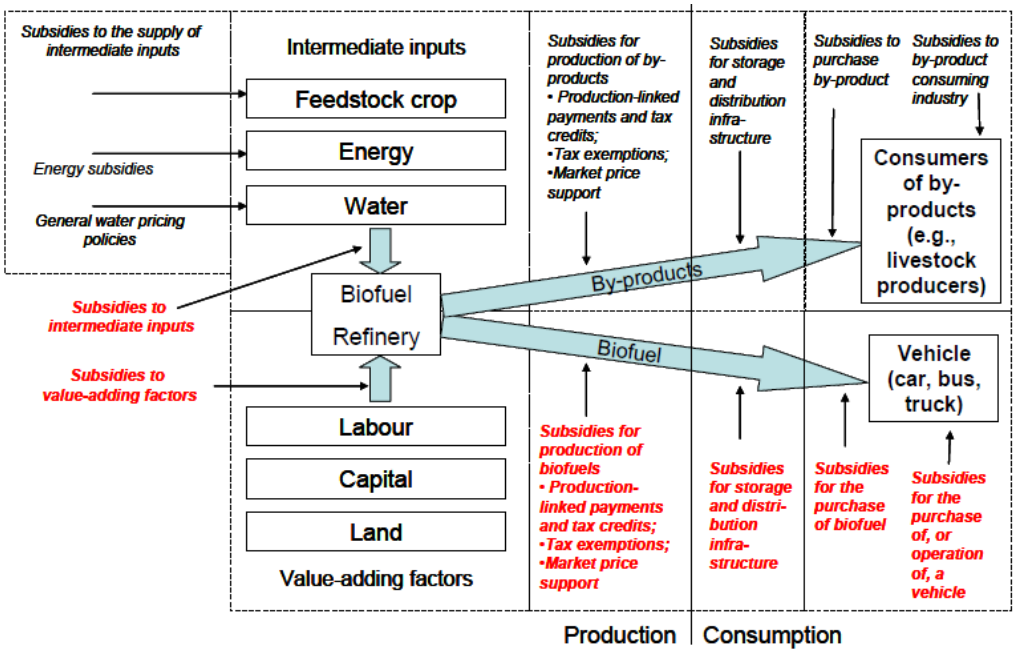

Figure 3.1. Subsidies in different phases of biofuel life cycle (GSI, 2008)

In 2011, renewable sources of energy (excluding hydro) received an estimated US$88 billion in global subsidies, with US$24 billion going to biofuels for transport; with the new policies this value should rise to US$185 billion in 2020 and US$240 billion per year by 2035 (OECD/IEA, 2012). According to the IISD report (2013), the subsides in biodiesel given by the EU were estimated to be around €5,000 million (US$5,480 million) in 2011 and will be around €7,900 million (US$8,660 million) in 2020, which represents an increase of 58% in 10 years (Charles et al., 2013).

Mainly because of these government interventions, the use of biodiesel has been significantly increasing over recent years (some of them are presented in Table 3.1) (Sorda et al., 2010) and several studies affirm that without these policies the targets for 2020 would not be met (Chang et al., 2012).

The EU, the US and Brazil were selected for this study because they are the three biggest producers in the world and all have established targets. Table 3.1 and Figure 3.2 present the increasing production and consumption of biodiesel after the creation of their biofuel policies (between 2000 and 2005).

Table 3.1. Biodiesel production and consumption in millions of litres by year (OECD-FAO, 2013)

Biodiesel Production					
	2004	2008	2012	2016*	2020*
European Union-27	1422.2	7382.2	10908.8	12980.8	17518.6
United States	283.9	1856.5	4232.1	5143.6	5849.0
Brazil	0.0	1167.2	2738.6	2822.4	3156.6
World	2301**	14574**	22400***	31706.4	38195.4
Biodiesel Consumption					
	2004	2008	2012	2016*	2020*
European Union-27	1219.8	10155.7	13980	16011.3	19594.5
United States	283	1095.7	3952	4865.3	5717.7
Brazil	0	1170.7	2739.2	2793.7	3107.8
World	..	..	..	31706.4	38195.4

*Estimated / **(Sorda et al., 2010) / ***(REN21, 2016)

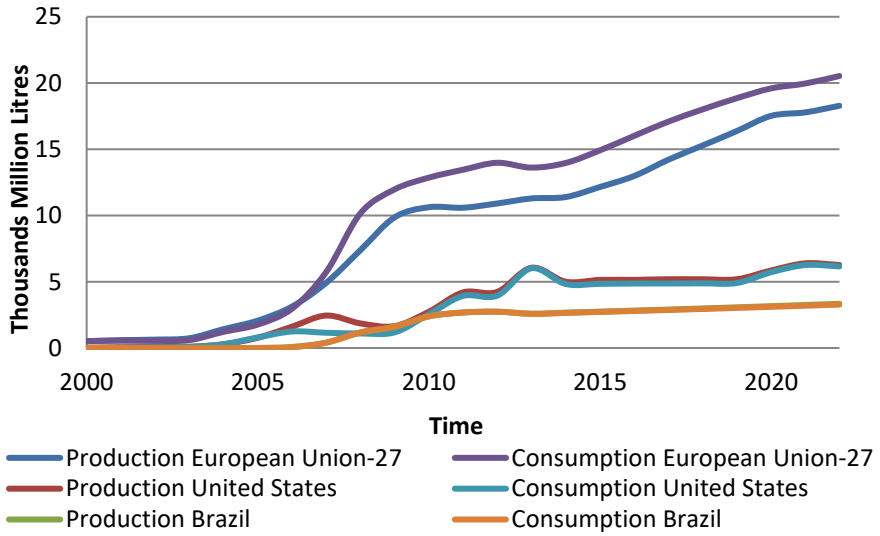

Figure 3.2. Biodiesel production and consumption of EU, United States and Brazil (OECD-FAO, 2013)

The selection of the zones studied was done based on their biodiesel production and world representativeness. However, it must be noted that there are also incentives and policies in action around the world.

3.3.1.1. Policies around the world

Currently diesel is the most consumed fuel in China, but biodiesel use has not been encouraged by the government (Qiu et al., 2012). The 2020 target is defined by a "Medium and Long-Term Development Plan for Renewable Energy" from 2007 and it is not ambitious: just 15% of renewables used in energy consumption, which includes a specific target of 2 million tonnes of biodiesel (Chang et al., 2012). Currently China is a major importer of vegetable oil, but biodiesel is produced principally from waste food oil and animal fat. The main official target refers to Jatropha cultivation designated area (GSI, 2008) and there are no direct subsidies or national standards (Sorda et al., 2010). Some recent studies demonstrate the potential of the country in developing algae biofuels in order to supply the energy necessary in the next 20 years (Zhou et al., 2013).

India has installed capacity greater than actual production. The relevant policies on biofuels started in the country in 2003 with the "Electricity Act" and the "National Biofuel Mission" that included a programme of ethanol blending. However the "National Action Plan on Climate Change" only came in 2008 followed by the "National Policy on Biofuels" that promoted the development of next generation biofuels and established the blending target of 20% by 2017 (Lohan et al., 2013, Singh and Setiawan, 2013). In India, oil seed plants could be

cultivated in wastelands and fallow land (Biswas and Pohit, 2013) and algae could be cultivated in flooded paddy fields (Chanakya et al., 2013).

Australia has a renewable energy target, subsides and public investments to increase the use of renewable energy (an amount of US$15.7 million is pledged for advanced biofuels research), but there are no national mandatory targets for the use of biofuels (REN21, 2016). In 2010 the "Alternative Fuels Taxation Policy" was launched which benefits biofuel development. Currently, the country imports more than 40% of biodiesel used and feedstock development researchers are taking places in Australian universities and researcher centres, including algae biodiesel in the University of Queensland (BAA, 2013).

3.3.1.2. European Union (EU)

The EU is the world's largest biodiesel producer (Germany and France leading) with 41% of total global production (REN21, 2016). Their policies in biofuels were driven by the high oil dependence of the EU's transport system (usually the oil was imported from Russia and "politically unstable regions" in the Middle East and Central Asia) and by the targets of the Kyoto Protocol (Afionis and Stringer, 2012).

There were 88 facilities identified with an installed capacity of 65 billion litres in the EU in 2013, but it is possible that there may be many more since there is no register or control of the producers and there are some small producers (Charles et al., 2013).

The European Climate Change Programme (ECCP) was created in 2000 with the paper “Towards a European Strategy for the Security of Energy Supply” (2001) (Acquaye et al., 2012). In 2003, a significant step was given with the statement of the Biofuels Directive that created targets of 2% of biofuel in petrol and diesel by 2005 and 5.75% by 2010. These targets were not achieved, with the exceptions of Germany, Sweden and Austria, so the directive was reviewed (Afionis and Stringer, 2012).

The Renewable Energy Directive (RED – EU Directive 2009/28/EC and amended as RED – EU Directive 2015/1513) was endorsed by the European Parliament with a target of 10% for biofuels in transport fuel by 2020. Adopting the LCA as a reference method, it means calculating the emission through the entire production chain and a GHG emissions reduction during product (fuel) life cycle of at least 35% (Sorda et al., 2010). This Directive counts the contribution of biofuels from lignocellulose material, residues and algae twice towards the targets as a way to promote these advanced fuels (OECD/IEA, 2013). Table 3.2 shows the most important six moments for EU policies.

Table 3.2. EU main policies in biofuels

Directive/Law	Year	Description / Target
European Climate Change Programme Towards a European Strategy for the Security of Energy Supply (COM(2001)370)	2000 2001	20% of biofuel in fuel production by 2020 / Future proposal: tax exemption
Directive on Biofuels for Transport (2003/30/EC)	2003	2% of biofuel in petrol and diesel by 2005 and 5.75% by 2010
Directive on the Taxation of Energy Products and Electricity (Directive 2004/75/EC)	2004	Taxation of energy products. It proposes exemptions and transition periods
EU Biofuels Strategy **Group of policy support and strategic planning:** • **European Council Action Plan (2007-2009)** • **Energy Policy for Europe** • **Renewable Energy Road Map - Renewable energies in the 21st century: building a more sustainable future** • **Strategic Energy Technology Plan (SET-Plan): Towards a low carbon future**	2006 2007	Proposed the target of 20% of renewable energy in energy consumption by 2020; 10% minimum use for biofuels and 20% reduction in the emission of greenhouse gases by 2020
Renewable Energy Directive (RED – EU Directive 2009/28/EC)	2009	10% of biofuels in transport fuel by 2020 / 35% less GHG emissions during life cycle
Renewable Energy Directive (RED – EU Directive 2015/1513)	2015	6% less GHG emissions per unit of energy of fuels / Less than 7% on agricultural land for energy

Beyond the EU Directive, each member state has its own policies and targets. One example is the UK Low Carbon Transition Plan (LCTP/2009) targeted to cut 18% of GHG emissions on 2008 levels by 2020 and over a third on 1990 levels over the same period (Acquaye et al., 2012) with a 10% of its transport fuel from renewable sources (House of Commons, 2016). The biggest targets in percentage of renewable energy in 2020 are from Sweden with 50% and Austria

with 45%, while countries like Malta and Luxemburg have targets lower than 12% (REN21, 2016).

A number of schemes for certifying biofuels have emerged since 2010 in order to give Renewable Energy Directive guidelines[1]. One example is the "International Sustainability and Carbon Certification" (ISCC) that is applied to ensure that GHG emissions are reduced. This explicitly requires that: land used to produce biomass should not be of high biodiversity and high carbon stock; good agricultural practices (protecting soil, water and air) are applied; and human rights, labour and land rights are respected (ISCC, 2014).

RED already stated that non-conventional biofuels, such algae biodiesel, should be encouraged by the Member States with investments in research and technology development (EU, 2009). One example of the initiatives raised to stimulate the research and innovation into advanced biofuel technologies is the "Leaders of Sustainable Biofuels" formed by technology developers and investors (LSB, 2013).

Another big investment in technology and research done by Innovation Union and Europe 2020 is Horizon 2020, a programme with a budget of €80 billion (around US$88 billion) available over 7 years, from 2014 to 2020, for several areas including energy, environmental and climate action, transport and others (EU,

[1] The sustainability schemes can be found in the EC website, at http://ec.europa.eu/energy/renewables/biofuels/sustainability_schemes_en.htm. Accessed 08/01/2013.

2014). In the energy area €3 million (US$3.3 million) was designated for new types of biofuel (engine tests and standards) to include those made from algae.

Biofuels Research Infrastructure for Sharing Knowledge (BRISK) initiated in 2011 with €10.84 million (US$12 million) and it is an initiative from the European Commission's 7th Framework Programme (FP7) to fund researchers in 33 experimental facilities around Europe (BRISK, 2014) such as the Algae Cluster which brings the three large scale algae biofuel projects together (BIOFAT, All-Gas and InteSusAl) (AlgaeCluster, 2016). Another example is EnAlgae that put together 19 partners in 7 EU member countries focusing on developing algae production technologies in order to decrease CO_2 emissions (EnAlgae, 2016).

Incentive programmes to develop algae biodiesel are also being run by each country and by private companies. One example is the construction of a 10-hectare plant, which is planned to be finished by 2017 in the South of France (Reuters, 2013)[2].

3.3.1.3. The United States (US)

In 2013 it was reported that the US uses 220 billion litres of diesel per year and according to EIA (2013) produces 3.6 billion litres per year[3] of biodiesel (a different value when compared to the OECD value of 4.2 billion litres and lower

[2] More programs are presented in http://www.biofuelstp.eu/algae-aquatic-biomass.html. Accessed in 15/10/2016.
[3] Table available in http://www.eia.gov/tools/faqs/faq.cfm?id=927&t=4. Accessed 15/10/2016.

than the standard requirement of 4.8 billion litres from the global renewable energy policy network (REN21, 2016)).

Policies to encourage the use of biofuels in the US started in 1978 with the "Energy Tax Act" that provided subsides to ethanol production. In 1990, ethanol was established as an additive for gasoline by the Clean Air Act. Table 3.3 illustrates the evolution of biofuels policies in the country.

Table 3.3. US main policies in biofuels

Directive/Law	Year	Description / Target
Energy Tax Act	1978	Subsides to ethanol production
Clean Air Act	1990	Ethanol was established as an additive for gasoline
Energy Policy Act of 1992 • **Clean Cities** • **National Biodiesel Education Program** • **Volumetric Ethanol Exercise Tax Credit**	 1992 1993 2002 2004	Reduce petroleum consumption by 2.5 billion gallons by 2020 / Invest in research to B20 (20% biodiesel in diesel) / Program to collect and disseminate biodiesel information / subsidy to gasoline blenders and to biodiesel produced
Renewable Fuel Standard (RFS and RFS2) Energy Independence and Security Act (EISA)	2005 2007	Standardisation and targets on LCA / production of 79 billion litres of biofuels (excluding ethanol from corn) by 2022/ 50% reduction in life-cycle GHG emissions
American Recovery and Reinvestment Act	2009	USD 80 billion to support research, development, and deployment. USD 30 billion is in the form of tax-based incentives
US Climate Action Plan	2013	USD 8 billion in loan to energy projects. Carbon pollution standards by EPA. 3 billion tons reduction in CO_2 emissions by 2030 and other goals to decrease the fossil fuels usage and pollutant emissions.

One important financial support for biofuels was enacted in 2004: the Volumetric Ethanol Exercise Tax Credit (VEETC) guarantees subsidies to gasoline blenders (US$0.51 per blend gallon used) and also to biodiesel produced (US$1 per gallon) (Sorda et al., 2010).

The Renewable Fuel Standard (RFS) that imposes the obligatory consumption of biofuels had its first version in 2005 and was revised in 2007 (RFS2), the same year of the "Energy Independence and Security Act" (EISA 2007). RFS2 included standardisation and goals on LCA (Philp et al., 2013) and the country set the target to produce 79 billion litres of biofuel (excluding ethanol from corn) by 2022. In addition, it stated that producers of advanced biofuels should reduce life-cycle GHG emissions by 50% compared to the one presented at the time (U.S., 2007).

A National Biodiesel Accreditation Programme (BQ-9000®), open to manufacturers, marketeers and distributors, was created to guarantee the quality during the entire life cycle of biodiesel production and to define ASTM standards for comercialised biodiesel (ASTM D6751) (NBAC, 2014).

In 2008, the Biomass Programme aimed to decrease gasoline consumption by 30% (from 2004 to 2030) and to make cellulosic ethanol, alternative light-duty and diesel replacement fuels (including algae-derived biofuels) (Sorda et al., 2010).

Algae and other biomass fuels are receiving investments from US government and the private sector. A research programme, which focuses on using synthetic genomic science (genetic modifications) to improve production, was announced by Synthetic Genomics Inc. with ExxonMobil (Abdelaziz et al., 2013b). The US Department of Energy announced an investment of US$13 million to accelerate the development of second and third generation biofuels and the Bioenergy Technologies Office released a programme to reduce the dependence on oil by developing alternative fuels including biofuels from algal biomass (U.S.DOE, 2010). There are also the Algal Biofuels Consortia, a public/private initiative in research with the cooperation of universities, national laboratories and industry (U.S.BETO, 2013) and the Algae Programme that focuses on the technical issues to promote feasable algae biofuels (U.S.BETO, 2016).

3.3.1.4. Brazil

Brazil is considered the most developed country in biofuels programmes. It started, following the oil crisis in 1970, with the National Fuel Ethanol Programme Pró-Álcool (in the Decree 76.593 of 1975) that stimulated Flex-Fuel cars manufacture to exploit sugar cane ethanol / gasoline blends (E25) and pure bioethanol. The programme was so successful that ethanol production increased almost fifty-fold from 594,985 m^3 (1975) to 27,604,120 m^3 (2011) (Stattman et al., 2013). Moreover 96% of automobiles sold in the country in 1985 used ethanol while more than 80% of the cars sold are Flex-Fuel; furthermore, since 2004 there has been no excise tax on ethanol fuel (Sorda et al., 2010).

The National Programme on Biodiesel Production and Usage (PNPB) was implemented in 2005 requiring a blending of 2% of biodiesel in petroleum diesel before 2012. In 2012, biodiesel production was more than 2.7 billion litres and the content of biodiesel to diesel was already 5% with an increase to 10% expected by 2019. Table 3.4 outlines the most important Brazilian actions in the development of biofuel use.

Table 3.4. Main Brazil policies on biofuels

Directive/Law	Year	Description / Target
National Fuel Ethanol Program Pró-Álcool (Decree 76.593)	1975	E25, pure ethanol and flex-fuel cars
National Program on Biodiesel Production and Usage (PNPB)	2005	2% of biodiesel in diesel before 2012
India-Brazil-South Africa Declaration on Clean Energy (Voluntary Agreement)	2007	Reduce CO2 emissions by between 36 and 39% by 2020
Brazil National Climate Change Plan	2008	Envisage 5% of biodiesel in 2010 rather than 2013
Mandatory Biodiesel Requirement	2010	Started with PNPB: 2008 – 2%; 2009 – 4%; and 2010 – 5% of biodiesel in diesel
Mandatory Biodiesel Requirement Law (13.033 and 13.263)	2014 2016	8% of biodiesel in diesel by 2017; 9% by 2018 and 10% by 2019.

According to the National Petroleum Agency (ANP, 2013) there were 70 biodiesel production plants operating with an output capacity of 23 million litres per day in 2013. The programme, coordinated by the Ministry of Mines and Energy (Ministério de Minas e Energia – MME), also includes tax incentives that vary from 73% to 100% of federal levy (Sorda et al., 2010), incentives to produce feedstocks other than soybean, to help generate regional development and crop

diversification, and incentives to the social inclusion of small-scale farmers (family farmers) (Stattman et al., 2013).

This social inclusion is ensured by the “green stamp” (“Selo Combustivel Social” or “Selo verde”) granted by the Ministry of Agricultural Development (Ministério do Desenvolvimento Agrário – MDA) to any biodiesel producer that meets the minimum percentage of feedstock purchase from family farms as defined in Ordinance number 60 of 2012 (Brasil, 2012). In 2010, the percentage of biodiesel feedsctock purchased by the producers with the stamp was 26% (MDA, 2011). Another advantage of this measure is the environmental sustainability guaranteed by the authorization of the land use by the regulatory agency to control the feedstock production.

The PNPB does not exclude the investment in various feedstocks, but the focus is on the oilseeds that can contribute to the development of all parts of the country (especially the northeast) and social improvement.

The diversification of raw materials is still far from being achieved and at the end of 2013 soy contributed to more than 75% of production, followed by cattle fat with 17%, while the biggest promises, such as castor beans and *Jatropha Curcas*, represented less than 2% (ANP, 2013).

A partnership between the Ministry of Science, Technology, Inovation and Comunication (MCTI), the National Council for Scientific and Technological Development (CNPq) and the Special Office of Fisheries and Aquaculture (SEPAq)

support projects with aquaculture and microalgae as a feedstock to biodiesel production (Brasil, 2008). It has already invested around R$26 millions (US$8.2 millions) in algae biodiesel research projects in the country (MCTI, 2015).

3.3.1.5. Evaluation of the policies situation

In these scenarios, with a lot of policies of climate change and renewable energy, it is possible to observe that the first barrier to achieving the targets is how to achieve the minimum percentage of renewable source fuel without deforestation and whilst keeping the sustainability of the biofuel production (Afionis and Stringer, 2012). It is therefore very important to consider the choice of the feedstock and the places of investments, since it can influence the other spheres of market such as food availability and price.

Another important point, especially in the EU, which is the only region in the cases studied that consumes significantly more biodiesel than it produces (as demonstrated in Table 3.1 and Figure 3.2), is the necessity to strengthen international trades, since biofuels could be imported from countries with more favourable conditions to feedstock production (such as climate). In 2007, Brazil tried to liberate trade by proposing the inclusion of biofuels as environmental goods, but the EU created sustainability standards that do not evaluate Brazilian biofuel as sustainable (based on the crop area) and the US do not discuss the subject to protect its internal market (Afionis and Stringer, 2012).

International Standards also should be created to facilitate international commerce. In the US the American Society for Testing and Materials (ASTM)

regulate standards such as ASTM D6751 for biodiesel while in Europe there is EN 14214 from the European Committee for Standardization, but they are not exactly the same which makes the comercialization of biodiesel more difficult. The differences include the minimum cetane number, maximum sulfur amount, maximum carbon residue and others.

Based on analysis of policies, it is noticeable that the government exercises a fundamental role in biofuels development and market, but it is not the only variable in the production (producers, consumers and trades are also involved): ambitious targets have not been reached and are constantly adjusted. It is predictable that policies about the environment and energy must go together. The creation of pollutant emissions limits, resource use restrictions and renewable energy use targets (including blending targets) should take into consideration feedstock availability, market shares and developed technology.

A hypothesis from DNV (2010) predicted a 2020 scenario where transportation fuel could have 10% of biofuels sold in blending. Algae had not been considered as feedstock. Even in recent studies, algae biodiesel production is usually treated as a new field that needs research and investment in order to develop the technology and become economically and environmental feasible. Currently there are just the first pilot plants and small commercial plants of algae production, most of which supply the chemical industry, so they are not considered to be contributing to the biofuel targets. An area availability

assessment was therefore carried out in order to understand how algae might participate in biodiesel production in the future.

3.3.2. Area assessment for algae cultivation to produce biodiesel

The area availability assessment for algae cultivation to produce biodiesel was calculated in three steps. First, the projection of how much biodiesel will be needed to be produced from algae by 2020 in order to (i) achieve the current targets and (ii) entirely replace diesel was determined. Secondly, the productivity of algae biodiesel per unit of cultivation area was determined, and subsequently, with the results of the two first steps, the land requirement to produce the necessary fuel was calculated. The objective of this analysis is not to suggest that the algae would be used to replace all the feedstock for biodiesel, but only highlight the potential of the use of this biomass.

3.3.2.1. Biodiesel Requirement

The amount of biodiesel required was calculated based on predictions for 2020. The first situation was based only on the targets that each region needs to achieve (as presented in Table 3.5) and the second was based on the total biodiesel necessary to replace the consumption of diesel (Table 3.6).

The targets were taken from the directives of each region and were used as production requirements. The biodiesel volume requirements by the targets are presented in Table 3.5.

Table 3.5. Biodiesel targets 2020

	Directive	Target	Annual Biodiesel Requirement 2020	Source
EU	EU Directive 2009/28/EC (EU, 2009)	10% of biofuel in transport by 2020	27 billion litres	(Pinto, 2011)
US	Energy Independence and Security Act (U.S., 2007)	79.5 billion litres of advanced biofuel by 2022	56.8 billion litres[a]	(U.S.DOE, 2010)
Brazil	Mandatory Biodiesel Requirement 2015 emended (Brasil, 2005)	B10 - Blending of 10% of biodiesel in diesel	7 billion litres	(Argus, 2011)

[a] Interpolated target considering only biodiesel by 2020

The total necessary production of algae biodiesel in 2020 to replace the use of fossil based diesel was calculated considering the following assumptions:

The biodiesel necessary to replace all diesel is the sum of the projections of diesel and biodiesel consumption in 2020 less the actual installed biodiesel capacity (since the objective is not to stop the use of existing sources of biodiesel, but to use algae to raise this production), given by:

$$Total = (Diesel\ consumption\ 2020 / 0.93) + Biodiesel\ consumption\ 2020 - Current\ installed\ capacity$$ **Equation 3.1**

Diesel consumption has been converted to biodiesel consumption based on the difference between the energy content in diesel and biodiesel: the factor

0.93 represents the fact that 100% biodiesel (B100) has 93% of the energy content as the same volume of diesel (U.S.DOE, 2013).

Installed Capacity is the current available production of biodiesel from all available feedstocks, so it does not include algae. There are a number of projections of diesel and biodiesel usages in 2020 in the public domain, and for this study those provided by the International Energy Agency (IEA) and by the Organisation for Economic Co-operation and Development (OECD) were considered.

It is acknowledged that the technological advances in fuel and engines around the energy economy can generate changes in diesel demand, but these were not considered in this analysis.

The data of diesel consumption were given in million metric tons of oil equivalent (mtoe) and were converted to litres based on 1000 litres of diesel being equivalent to 0.98 toe (OECD/IEA, 2012). Table 3.6 displays the outputs of the calculations.

Table 3.6. Total required biodiesel to replace all diesel (billion litres per year)

	Diesel consumption forecast 2020 [a]	Biodiesel consumption forecast 2020 [b]	Installed Capacity [c]	Required Biodiesel [d]
EU	267.96	19.59	9.1	298.62
US	196.43	5.72	3.6	213.33
Brazil	60.20	3.11	8.4	59.44
World	1494.9	38.20	22.5	1623.11

[a] Forecast from World Energy Outlook – IEA / Conversion based on 1000 litres = 0.98 toe
[b] Forecast from (OECD-FAO, 2013)
[c] World and EU (REN21, 2016); US (EIA, 2013); Brazil (ANP, 2013)
[d] Calculated from Equation 3.1

3.3.2.2. Production adopted

The oil produced by algae is affected by diverse factors, such as the species of algae, geography of facility location, cultivation and the oil extraction methods used.

Biodiesel production from algae is calculated using the following equation which is adapted from Sudhakar and Premalatha (2012):

$$BP = BM \times LE \times PE \times \frac{1}{\rho} \times n \times c \qquad \textbf{Equation 3.2}$$

Where:

- BP: Biodiesel production ($L \cdot ha^{-1} \cdot year^{-1}$);
- BM: Dry biomass production per day ($g \cdot m^{-2} \cdot d^{-1}$);
- LE: Lipid extracted (% wt. from dry biomass);
- PE: Process efficiency (%);
- ρ: Algae oil density ($kg \cdot m^{-3}$);
- n: Number of productive days per year – in ponds, photobioreactors, lake or sea ($days \cdot year^{-1}$);
- c: Factor to correct inconsistancies in units of mass and area = 10 $kg \cdot m^2 \cdot g^{-1} \cdot ha^{-1}$.

The productivity of biomass can be influenced by light exposure, algae strain, media and cultivation method. The intention of this study is to provide an outline for different locations and scenarios and does not focus on specific experimental parameters; the productivity values were based on cultivation methods available in the open literature. The most usual production method is an open pond raceway that gives between 20 to 30 $g \cdot m^{-2} \cdot d^{-1}$ of dry biomass (Collet

et al., 2014b). Some authors also use this value in Photobioreactors (PBR) (Jorquera et al., 2010, Davis et al., 2011), but there are some predictions with much higher production rates for PBR achieving 100 $g \cdot m^{-2} \cdot d^{-1}$ to more than 500 $g \cdot m^{-2} \cdot d^{-1}$ (Batan et al., 2010, Brentner et al., 2011). The most pessimistic scenario reports a productivity of just 3 $g \cdot m^{-2} \cdot d^{-1}$ (Passell et al., 2013) based on small cultivations done with the currently available technology in the market (open pond). All these quantities are given on dry mass bases. This review agrees with that of Collet et al. (2014b) where they conclude that the growth rate of microalgae can vary from 25 to 40.6 $g \cdot m^{-2} \cdot d^{-1}$ in open ponds (OP) based on 300 days of operation per year.

The cultivation of macroalgae was also studied and it indicates a similar productivity when compared to microalgae. Dibenedetto (2011) illustrated a scenario between 4 and 95 $g \cdot m^{-2} \cdot d^{-1}$ based on a culture of 7 or 8 months.

In light of this, this present study considered some cases of production for macroalgae and microalgae:

1. Low productivity of OP and macroalgae: 3 $g \cdot m^{-2} \cdot d^{-1}$;
2. Average productivity in OP and macroalgae: 25 $g \cdot m^{-2} \cdot d^{-1}$;
3. High productivity in OP and macroalgae: 50 $g \cdot m^{-2} \cdot d^{-1}$;
4. PBR productivity: 100 $g \cdot m^{-2} \cdot d^{-1}$.

These scenarios cover the range of productivity found in the literature and exclude outlier scenarios over 100 $g \cdot m^{-2} \cdot d^{-1}$ because these were considered overestimations.

The lipids content in the microalgae species cultivated to maximize lipids can vary from 20 to 50% wt. and is typically between 30 and 40% wt. of the dry mass. Some authors report however that the maximum amount of oil that can be extracted by current available techniques is 20% of the total dry mass (Sudhakar and Premalatha, 2012, Brune et al., 2009). There are currently investments in bioscience research to elevate this percentage, and as a consequence this study is considering three situations of percentage of oil extracted: 20, 30 and 40% wt. For some species of macroalgae; for example *Dictyota bartayresii*, *Spatoglossum macrodontum*, and *Dictyota dichotoma*; this percentage can be even smaller, around 10% wt. (Gosch et al., 2012), so this scenario was also evaluated. In all these cases, algae oil density was considered to be 850 $kg \cdot m^{-3}$.

The biodiesel conversion process is assumed to be the same as that currently achieved with other feedstocks in industry and it usually has an efficiency of 90%, however there are some techniques that are able to convert 100% (Collet et al., 2014a, Ventura et al., 2013). With this in mind three possible process efficiencies (including all steps) were assumed:

- Low efficiency: 80%;
- Medium efficiency: 90%;
- High efficiency: 100%.

The time available for production depends on the chosen system and the facility location. Tropical locations can have a longer cultivation time throughout the year than regions with higher latitudes since these latter areas have harsher

winters and larger variance in temperature. This study was based on an average available time for microalgae growth of 300 days, which is in-line with studies that adopted between 240 days (Pate et al., 2011) to the complete year (Collet et al., 2014a). The cultivation period for macroalgae was 210 days in accordance with the literature (Dibenedetto, 2011).

Based on Equation 3.2 and the assumptions described above, it was possible to calculate 48 scenarios which are presented in Table 3.7. Four subdivisions of oil content are considered: for macroalgae 10% of oil dry mass basis and and for microalgae 20, 30 and 40% dry mass basis.

According to these calculations and within the limits suggested, biodiesel productivity could vary from 593 to 24,706 $L \cdot ha^{-1} \cdot year^{-1}$ for macroalgae and from 1,694 to 141,176 $L \cdot ha^{-1} \cdot year^{-1}$ for microalgae.

Table 3.7. Scenarios of biodiesel productivity ($L \cdot ha^{-1} \cdot year^{-1}$)

		Macroalgae			**Microalgae**		
Oil content		**10%**			**20%**		
Days of productivity		**210 days**			**300 days**		
Process efficiency		**80%**	**90%**	**100%**	**80%**	**90%**	**100%**
Biomass productivity ($g \cdot m^{-2} \cdot d^{-1}$)	3	593	667	741	1,694	1,906	2,118
	25	4,941	5,559	6,176	14,118	15,882	17,647
	50	9,882	11,118	12,353	28,235	31,765	35,294
	100	19,765	22,235	24,706	56,471	63,529	70,588
		Microalgae			**Microalgae**		
Oil content		**30%**			**40%**		
Days of productivity		**300 days**			**300 days**		
Process efficiency		**80%**	**90%**	**100%**	**80%**	**90%**	**100%**
Biomass productivity ($g \cdot m^{-2} \cdot d^{-1}$)	3	2,541	2,859	3,176	3,388	3,812	4,235
	25	21,176	23,824	26,471	28,235	31,765	35,294
	50	42,353	47,647	52,941	56,471	63,529	70,588
	100	84,706	95,294	105,882	112,941	127,059	141,176

The most pessimistic scenario found in the literature was 11,300 $L \cdot ha^{-1} \cdot year^{-1}$, based on 20% wt. oil content (Brune et al., 2009); but the calculations presented here indicate even lower productivity when considering current technologies (cultivation productivity of 3 $g \cdot m^{-2} \cdot d^{-1}$ and 80% efficiency in the downstream process) and with the same oil content (20% wt.) reaching only 1,700 $L \cdot ha^{-1} \cdot year^{-1}$.

The most optimistic scenario found in the literature was for 136,900 $L \cdot ha^{-1} \cdot year^{-1}$ based on 70% wt. oil content (Chisti, 2007), which is smaller, while of a similar order of magnitude, than the values reached by this study for PBR (141,200 $L \cdot ha^{-1} \cdot year^{-1}$ for 40% wt. oil content). The discrepancy in values can be explained by varying biomass productivity adopted for the PBR, showing that the use of PBR for algae cultivation could have a high influence in productivity.

A theoretical assessment was reported by Weyer et al. (2010) which considered data for six global climates and a lipid content of 50% wt. If 90% of this oil is considered to be converted to biodiesel, the productivity at a site in Malaga in Spain would be 41,400 $L \cdot ha^{-1} \cdot year^{-1}$. The productivity of other regions in the study ranges from 40,700 $L \cdot ha^{-1} \cdot year^{-1}$ (Malaysia) to 53,200 $L \cdot ha^{-1} \cdot year^{-1}$ (Phoenix, US). The data in this work also agree with those found in other studies (that use lower lipid content), such as 12,000–98,500 $L \cdot ha^{-1} \cdot year^{-1}$ (Schenk et al., 2008), 58,700–136,900 $L \cdot ha^{-1} \cdot year^{-1}$ (Chisti, 2007) and 25,000–65,000 $L \cdot ha^{-1} \cdot year^{-1}$ (Sudhakar and Premalatha, 2012).

Considering the range in the productivity presented here and in the other studies, seven scenarios were assumed for further consideration, including one scenario for macroalgae and six for microalgae cultivated in open ponds and photobioreactors. They were chosen based on the rounded average, minimum and maximum productivities presented in Table 3.7 in order to embrace the most adopted values (base) and the most extreme situations (pessimistic and optimistic scenarios). The macroalgae scenario does not include the pessimistic and optimistic values because they are similar to the open ponds productivity. The seven selected scenarios are:

- Macroalgae: 12,000 $L \cdot ha^{-1} \cdot year^{-1}$;
- OP Pessimistic: 1,700 $L \cdot ha^{-1} \cdot year^{-1}$;
- OP Base: 30,000 $L \cdot ha^{-1} \cdot year^{-1}$;
- OP Optimistic: 70,000 $L \cdot ha^{-1} \cdot year^{-1}$;
- PBR Pessimistic: 20,000 $L \cdot ha^{-1} \cdot year^{-1}$;
- PBR Base: 80,000 $L \cdot ha^{-1} \cdot year^{-1}$;
- PBR Optimistic: 140,000 $L \cdot ha^{-1} \cdot year^{-1}$.

3.3.2.3. Area Requirement Discussion

The cultivation land required to achieve the targets and to replace all diesel consumption using the seven scenarios were calculated by the division of the biodiesel requirement (Table 3.6 and Table 3.7) by the theoretical production of biodiesel. The results are presented in Table 3.8 and Table 3.9.

Table 3.8. Area requirement (km^2) to achieve the targets (2020)

	Macro Algae	Microalgae – Open Pond			Microalgae – Photobioreactor		
		Pessimistic	Base	Optimistic	Pessimistic	Base	Optimistic
EU	22,500	158,824	9,000	3,857	13,500	3,375	1,929
US	47,333	334,118	18,933	8,114	28,400	7,100	4,057
Brazil	5,834	41,177	2,334	1,000	3,500	875	500

Table 3.9. Area requirement (km^2) to replace all diesel consumption in 2020

	Macro Algae	Microalgae – Open Pond			Microalgae – Photobioreactor		
		Pessimistic	Base	Optimistic	Pessimistic	Base	Optimistic
EU	248,850	1,756,588	99,540	42,660	149,310	37,328	21,330
US	177,775	1,254,882	71,110	30,476	106,665	26,666	15,238
Brazil	49,533	349,647	19,813	8,491	29,720	7,430	4,246
World	1,352,592	9,547,706	541,037	231,873	811,555	202,889	115,936

Clearly the range of values demonstrates the influence of each assumption on the final result. The three regions were studied separately because the impacts (social and environmental) change according to the distance between producer and consumer; this opens an opportunity for further study on each region.

As a general observation, the cultivation area requirement to achieve the current targets proposed by the policies of each region is attainable. Even in the most pessimistic scenario (pessimistic open pond cultivation), the maximum requirement to achieve the targets of the EU is 158,800 km^2 which represents 3.6% of the European land area (4,132,472 km^2); a similar observation is seen for the US which needs only 3.4% (334,100 km^2 out of a total of 9,826,675 km^2) and for Brazil that requires less than 1% of its total land (8,514,877 km^2)[4].

[4] The total area of each country varies according to the source. For this study data was used from the CIA. 2014. *The World Factbook* [Online]. Available:

It is important to consider that technology will improve and will therefore increase productivity, making algae more viable as a fuel source. A further study is recommended which considers projections and developments in engines and fuels. The most optimistic scenario for photobioreactors looks promising, and in all cases requires less than 0.5% of the total land of the respective regions. Even the pessimistic scenario represents a viable land requirement, therefore the potential of this cultivation technology to contribute to future biodiesel production is evident.

Another consideration is that photobioreactors can be installed offshore using technologies such as OMEGA – Offshore Membrane Enclosures for Growing Algae (Wiley et al., 2013, Harris et al., 2013) and this way the cultivation area would be outside of the land area. The same situation arises with the macroalgae that can be cultivated in fish farms (Roberts and Upham, 2012).

Analysing the microalgae in open ponds, the values found agree with other authors. Shirvani et al. (2011) determined that the area required to replace all diesel consumption in the world would be approximately 573,000 km^2 and Batan et al. (2010) indicated 477,000 km^2 compared to the base open pond case of this study of 541,037 km^2.

The open pond scenario was selected as a basis for the assessment calculations as it is the most developed technology available and the

https://www.cia.gov/library/publications/the-world-factbook/rankorder/2147rank.html [Accessed 15/03 2014]. and IBGE 2014. Paises@.

photobioreactor scenarios were considered to be overestimated by the authors due to the technological limitations to scale-up related to upstream process issues, such as keeping the production rate constant. The open pond cultivation approach has currently been shown to be able to produce 30,000 $L \cdot ha^{-1} \cdot year^{-1}$ (Batan et al., 2010) and this value is expected to increase. Once PBR have reached large scale manufacturing then the assessment could also be made for such systems.

On the basis of this, areas of 9,000 km^2 for the EU, 18,900 km^2 for the US and 2,300 km^2 for Brazil will be required to produce the biodiesel volume established by their targets. While to replace fossil-diesel use, land requirements could be: 99,500 km^2 for the EU, 71,100 km^2 for the US, 19,800 km^2 for Brazil and 541,000 km^2 for total global consumption replacement (Table 3.8 and Table 3.9). These numbers are not unfeasible when we compare them with plantations of soy, for example, which covers 1,087,000 km^2 around the world, with 277,000 km^2 being located just in Brazil with around 25% destinated to fuel (Embrapa, 2014).

Current installed biodiesel facilities (using other feedstocks) can produce up to 0.4 billion litres per year per facility, so in order to meet the current targets it would be necessary to construct more than 4,050 new facilities like these current facilities in the world. Locally the EU would need approximately 747 new units, the US would need 533 and Brazil should construct 150 new facilities. At 30,000 $L \cdot ha^{-1} \cdot year^{-1}$ this represents a cultivation area of 140 km^2 per installed

facility and a CO_2 input of 2.5 x 10^9 kg·year^{-1} which is less than the emission of one power plant in the US (approximately 3.8 x 10^9 kg·year^{-1}, assuming a capacity of 600 MW, capacity factor of 65% and emission rate of 1.1 kg CO_2·kWh^{-1} (EPA, 2014)). It would imply that the larger the power plant, the higher the algae productivity which could be attained so area requirements could be reduced.

Besides the cultivation land, it is important to include space for treatment and the conversion process. Zaimes and Khanna (2013) posited that a cultivation area of 5 km^2 would require an infrastructure of the same size, while Campbell et al. (2011) used 1 km^2 of buildings to 4 km^2 of ponds. This implies a minimum of 35 km^2 for additional process per facility which seems very high but is likely to come down significantly with scale. This study proposes that two-thirds of the facility area should be destined for cultivation and one-third occupied with other infrastructure.

In order to better illustrate these scenarios, Table 3.10 presents a comparative study of the areas required for each case considering the base case production of biodiesel from microalgae in open pond. As demonstrated in Table 3.10 it would be necessary to use a land area equivalent to approximately half of Italy to cultivate the necessary algae to replace all diesel consumption in the EU.

Table 3.10. Comparative requested area including facilities (base case: microalgae in open pond)

	Total area [a] (km^2)	Necessary Land to achieve the targets [b]		Necessary Land to replace all diesel consumption [b]		Comparative area scale to replace diesel consumption (approx.)
		Area (km^2)	Percentage (%)	Area (km^2)	Percentage (%)	
EU	4,132,472	13,500	0.33	149,310	3.61	1/2 Italy
US	9,826,675	28,400	0.29	106,665	1.09	Tennessee State
Brazil	8,514,877	3,500	0.04	29,720	0.35	2/3 Rio de Janeiro State
World	148,940,000	-	-	811,555	0.54	1/10 Brazil

[a] Continental Area provided by (CIA, 2014) and (IBGE, 2014).

[b] Applying factor 1.5 because of the infrastructure space requirement.

3.3.3. Location of the facilities

After the calculation of how much area is necessary to cultivate algae to produce biodiesel, it is necessary to assess the available area.

The area to cultivate the algae should be selected to meet the following conditions (Kovacevic and Wesseler, 2010):

- Favourable climate:
  - Solar radiation – mostly sunny all year long;
  - Weather – constant temperature mostly between 20 and 30°C (Chisti, 2007).
- Available area;
  - Onshore:
    - Flat land;
    - Empty – not cultivated, protected or occupied by cities or pastures;
  - Offshore:
    - Available sea space close to the coast;
- Proximity to resources;
  - Water source – fresh, sea water or wastewater.

- Nutrients source – fertilizers or wastewater;
- Carbon source – power plants or industries.

Separate analyses were done for each of these necessary conditions for each case study to replace the use of diesel with biodiesel.

3.3.3.1. Climate

There are some divergences in the methodologies of world climate characterizations. While not selecting the specific species of algae, it is possible to make some gross observations about the limiting climate in algae cultivation considering different approaches.

Two examples are presented in Figure 3.3. The first one is Köppen-Geiger classification defined by Kottek et al. (2006) where the ideal climate would be equatorial, arid or warm temperate with low variation of temperature during the year. For the EU case, the favorable conditions in climate are found in the south of the region, mostly around 40° north latitude, in countries such as Portugal, Spain, Italy and Greece; whereas the US has the best conditions in the southwest of the country and Brazil in the northeast and centre. Another example is the IPCC classification used by Harmelen and Oonk (2006) that is based on the annual average temperature. In their study the possible cultivation area was delimited by the latitude, up to 37° north and south (represented by the area inside the rectangle in Figure 3.3).

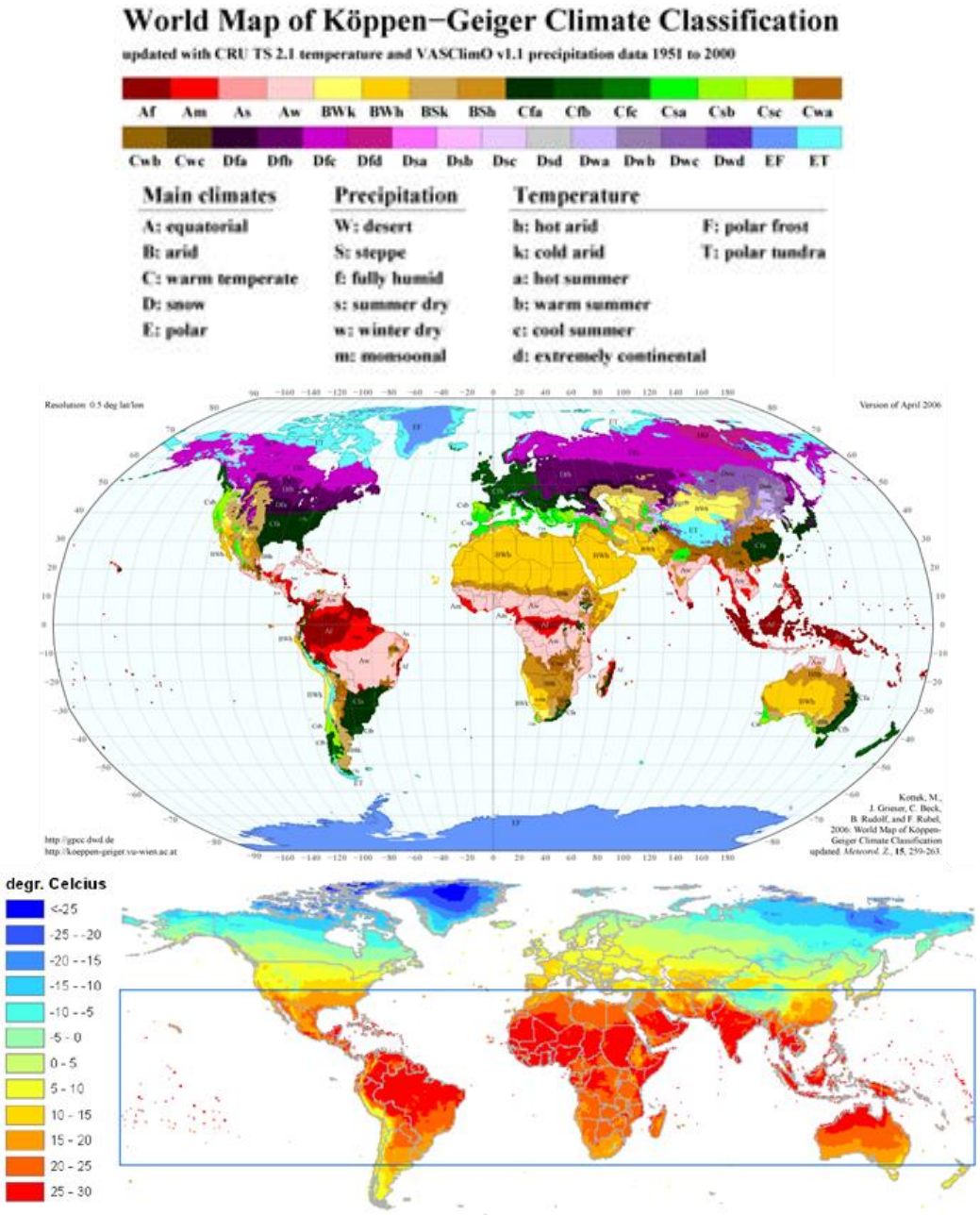

Figure 3.3. Köppen-Geiger climate classification (Kottek et al., 2006) and IPCC classification (Harmelen and Oonk, 2006)

This study, on the other hand, chose to use a classification based on the minimum and maximum temperature during the year in order to locate the areas with small temperature variations which are more favourable for algae cultivation. The acceptable minimum temperature for cultivation is between 10

and 35°C and the maximum between 15 and 40°C; on an annual basis the temperature should not be outside these limits, as if this occurs the land is considered incompatible for algae production. Figure 3.4 illustrates the land areas excluded from cultivation (in red) considering the monthly temperature range described above.

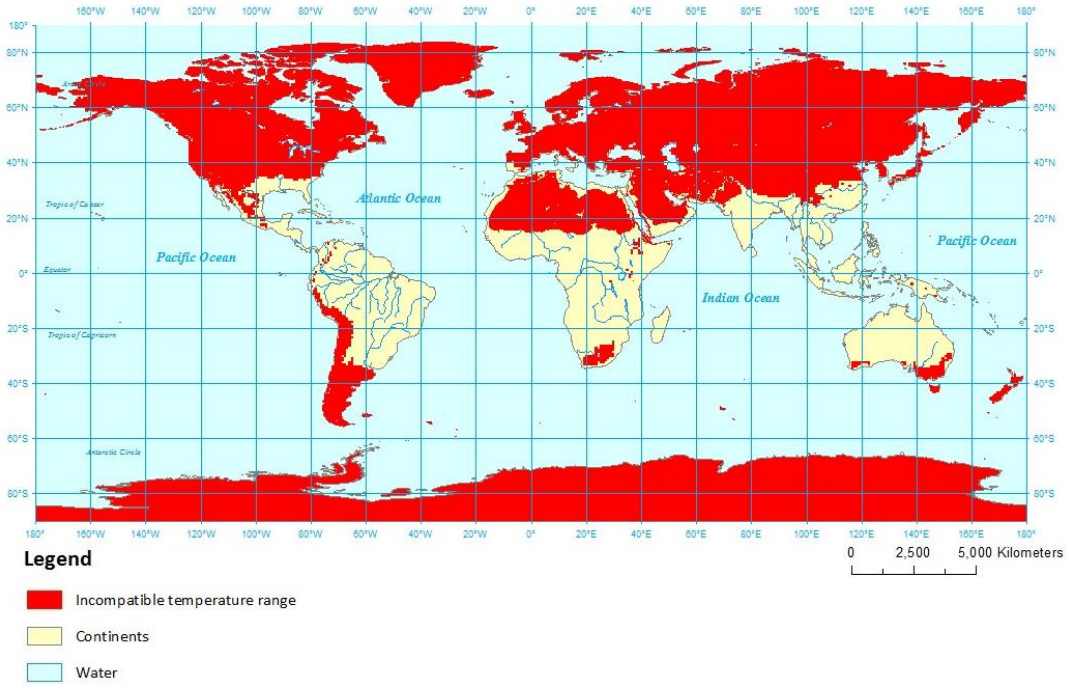

Figure 3.4. Onshore area with incompatible temperature to cultivate algae (min between 10°C and 35°C and max between 15°C and 40°C) (ArcGis-Online, 2014)

In light of the temperature range, it is possible to observe that there is an unfavourable climate in Europe, however possible facilities could be located in the south of Portugal, Spain, Italy and Greece. The south of the US and all of Brazil also meet the temperature requirements and because of this these areas would be favourable locations for new cultivation areas. This analysis agrees with the IPCC classification discussed in Harmelen and Oonk (2006). Shorter growing

seasons can be considered in locations where the cultivation temperature ranges are achievable for some of the year, but are not covered in this analysis due to the availability of data.

This analysis must be further detailed after a selection of the algae species, because there are species able to grow under colder or hotter temperatures and also because there is the possibility of cultivation in shorter periods of the year. An evidence of this is the cultivation of algae *Chlorella vulgaris* in the United Kingdom proposed by Stephenson et al. (2010), but it is not the focus of this research.

3.3.3.2. Available Area

The available area to cultivate algae should be assessed in order to not increase the impacts of land use change. Onshore, cultivation must be done on flat land to make the construction of open ponds or photobioreactors easier. Offshore, using floating photobioreactors or growing macroalgae, the area should be close to the coast to enable the transportation of raw material and products. The facility, when cultivation is carried out on land, should be allocated in a flat area that is not cultivated, protected or occupied by dense population (urban).

In light of this, the available land area was calculated based on the current soil occupation. The calculation and assumptions are summarized in Table 3.11 and in Figure 3.5. The calculation was based on the total area and it is interesting to note that the available land is much larger than that required as presented in Table 3.10.

Table 3.11. Available land area calculated by occupation of the soil (km²)

	EU	US	Brazil	World
Total Area [a]	4,132,472	9,826,675	8,514,877	148,940,000
Urban Area [b]	102,478	112,197	40,469	605,875
Protected Area [c]	615,517	1,342,324	2,213,868	18,915,380
Cultivated Area [c]	1,167,668	917,811	795,290	49,116,314
Permanent Pasture Area [c]	580,037	2,276,841	1,972,897	33,586,546
Available Land area	1,957,074	5,177,502	3,492,353	46,715,885
Current Biodiesel Targets (%[d])	0.69	0.55	0.05	-
Replacement of Diesel (%[d])	7.63	2.06	0.85	1.74

[a] Continental Area (soil) (CIA, 2014) and (IBGE, 2014)

[b] Urban area projection (Angel et al., 2010)

[c] (IBGE, 2014)

[d] % of the available land required to produce biodiesel

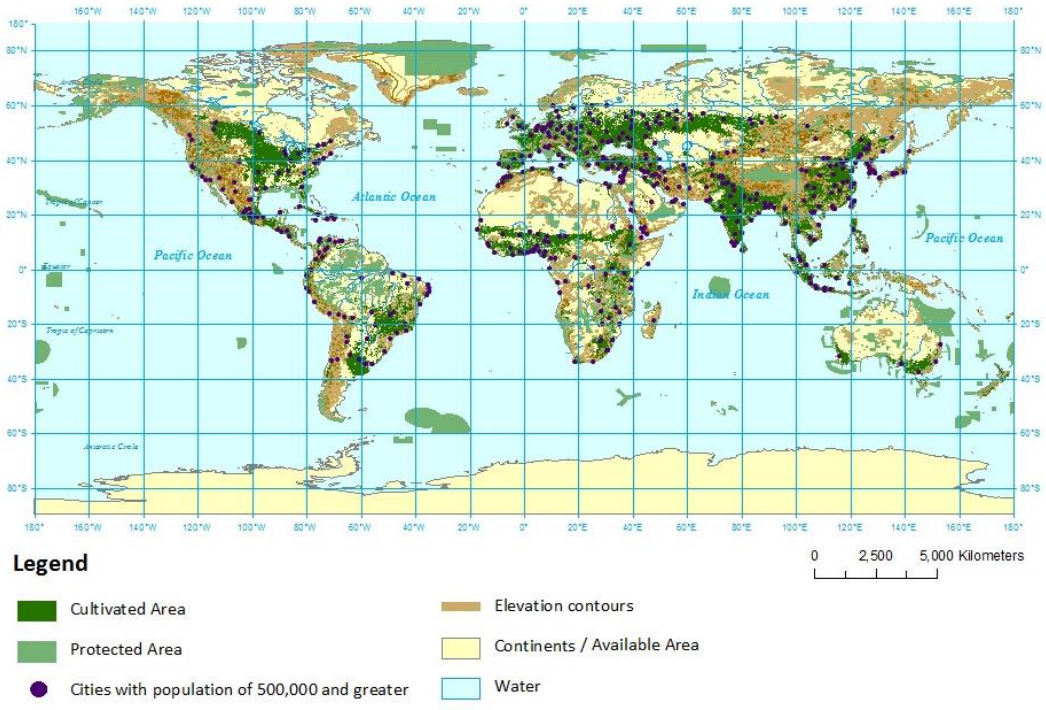

Figure 3.5. Occupied and available area (ArcGis-Online and Protectedplanet, 2014)

From the results of the occupied area (Figure 3.5) it is possible to do some critical observations. Although the data used for the map creation were the best available for a global resolution, the protected areas should include some zones that are unprotected, such as parts of the Amazon Rainforest or high slope areas. Other limitations are the methodology used to map the cultivated area by

percentage of agricultural land and presenting an overestimated result that covers almost all of Europe. A final caution should be considered with regards to urban areas as this was only illustrated by the location of cities with more than 500,000 habitants. Nevertheless, from Figure 3.5, it is possible to deduce that there are a great number of flat areas available to cultivate algae, but there are already highly occupied areas with cultivation, so it would be pertinent to consider offshore production.

Interestingly, there are relevant differences in the calculated (Table 3.11) and mapped (Figure 3.5) areas. It demonstrates the divergences in the data sources and the necessity of better global data references.

3.3.3.3. Proximity to the Resources

Besides climate and space, algae cultivation also needs to have resources available; for example: a sea as a water source, wastewater as a source of nutrients and a power plants as carbon source. The facility's construction is limited by the proximity to these resources because the price and energy to acquire and transport them can make the process unfeasible.

The water source is easily solved by building plants close to the coast and close to wastewater treatment plants; this is the most feasible option as they can provide water and nutrients at the same time. For this study, the facility should be close to well-populated urban areas (>500,000 inhabitants) since these locations already have wastewater treatment plants and the necessary infrastructure to provide the nutrient inputs. Additionally, proximity to the coast

is required for saltwater species. It is important to remember that the proximity to cities is a requirement for feasibility and the necessary area to achieve the required productivity would be around 140 km^2. It is suggested that further work considers the feasibility of plants at further distances and more remote locations where transportation factors should be considered.

Power plants and industries must be used as the carbon source; this also generates an environmental advantage as the algae cultivation would capture the CO_2 emitted by them, the equivalent of 1.8 kg of CO_2 per kg of dry algae biomass (Chisti, 2007). Figure 3.6 illustrates the location of coal and gas power plants in the south of the EU, US and Brazil from 5 MW to 4,000 MW capacity in September 2014 (GEO, 2014); and consequently locations where algae biodiesel facilities could be constructed.

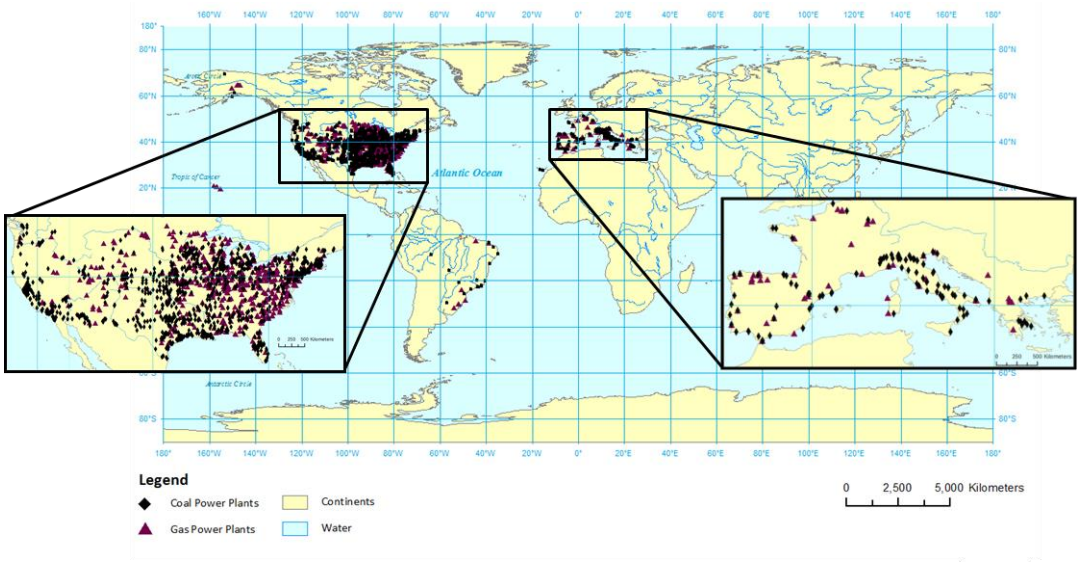

Figure 3.6. Coal and gas power plant location at the EU, US and Brazil in 2014 (GEO, 2014)

The EU has a considerable number of power plants able to tend the necessities of algae cultivation. Units that emit CO_2 are located in the Portuguese and Spanish coasts and in Italy and Greece. The US has more than 2,000 mapped power plants, with many concentrated from the centre to the east of the country and on the southwest coast. Brazil has a few mapped units (27) along the coast. However, one limitation of this methodology is that it does not consider all industries that emit CO_2, but only coal and gas power plants; so this consideration must be interpreted with caution. An example of this weakness is the Brazil case where the energy grid is based on hydroelectric plants and so there is not so many coal and gas power plants.

Other important considerations are possible future changes in the power grid, increasing the renewable source of energy, and the development of power plants resulting in a decrease of CO_2 emissions. In which case, the CO_2 would need to be be supplied from other industries or even in pure form which would require up to 85% less energy to pump (Campbell et al., 2011), however it is more expensive and not count as CO_2 sequestration.

It is worth noting that the proximity of inputs is more relevant to a specific local analysis since these data are not precise on global scale.

3.3.3.4. Superposition of the conditions

A superposition of the maps in Figure 3.4, Figure 3.5 and Figure 3.6 enables the areas that meets the climate, availability and proximity to the inputs

requirements to be obtained and gives the available areas to cultivate algae (Figure 3.7).

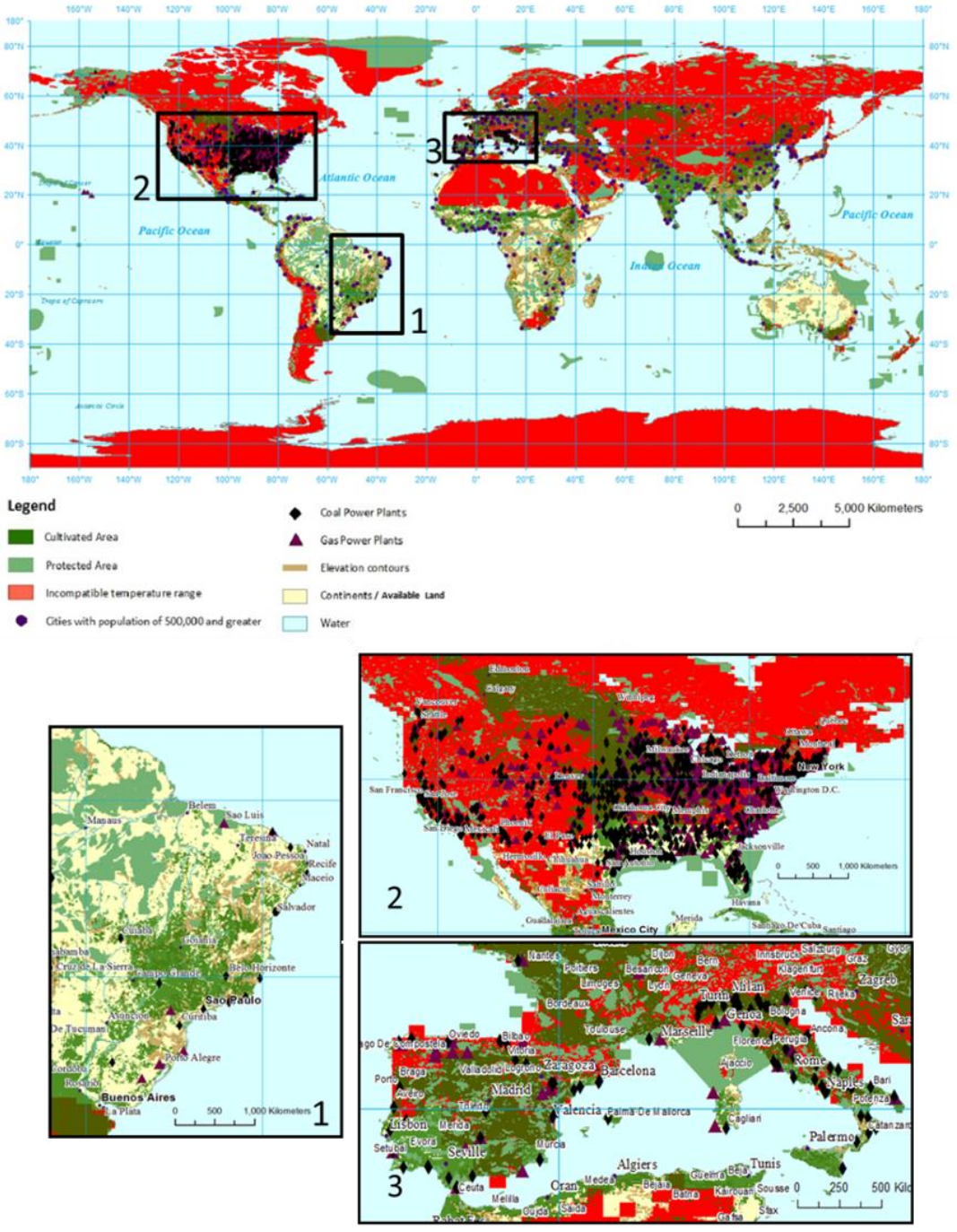

Figure 3.7. Available area – superposition of previous maps

The present findings illustrate possible locations to algae cultivation in the three case studied. In the EU, there are areas available in the southwest of Spain and south of Portugal, Italy and Greece; in the US, in south east of the country (especially from Texas to South Carolina) and along the coast for Brazil.

The findings of this study show that there is land area available to cultivate algae in order to achieve targets in the three case studied. Complete diesel replacement in Brazil and the US can be achieved through the construction of open ponds with a production of 30,000 $L \cdot ha^{-1} \cdot year^{-1}$ (a value expected to be reached in all locations). However space is often at a premium and is subject to land-use competition/constraints. For example, the EU may need to use offshore space and also select species that support cooler temperatures; other solutions could improve the upstream and downstream processes (including reactor design) to increase productivity and algae biodiesel produced in other locations where the climate and resources are favourable could be imported.

Following this analysis, it is possible to suggest some cities where the algae biodiesel facilities could be built in places that meet all requirements specified before. A number of cities with more than 100,000 inhabitants that match the requirements presented before were identified. These cities are presented in Table 3.12.

These results are consistent with some presented in previous research that makes global resource assessment analysis (Harmelen and Oonk, 2006) and where southwestern and eastern Spain and southeastern Italy were presented as

feasible sites in the EU (Kovacevic and Wesseler, 2010). However this research makes a more detailed mapped analysis, including data of protected and cultivated areas and proposing specific cities where facility construction is possible nearby.

Table 3.12. Suggestion of cities with more than 500,000 inhabitants where algae production the facilities could be located nearby

Supplied Region	Cities with more than 500,000 inhabitants	Number of Cities with more than 100,000 inhabitants
EU	Lisbon, Barcelona, Malaga, Marseilles, Genoa, Naples and Athens	32
US	Houston and Jacksonville	30
Brazil	São Luis, Fortaleza, Recife, Jaboatão dos Guararapes, Salvador, Feira de Santana, Rio de Janeiro, São Gonçalo, Duque de Caxias, Nova Iguaçu, São Paulo Metropolitan Area, Curitiba e Porto Alegre	52

3.4. Conclusions

The main policies and targets of the study areas (EU, US and Brazil) were analysed and it was possible to observe that the government have been failing to achieve the most challenging targets in decreasing CO_2 emissions and including renewable fuels in the transportation matrix.

International trade and feedstock diversification should be encouraged and algae biodiesel could play an important role in this field due its high productivity and the non-necessity of arable land. Based on the area requirement, this study was able to highlight the potential of algae biodiesel.

The area was calculated assuming the 2020 biodiesel demand to achieve the targets stipulated in government policies and to replace fossil-diesel, and considering different algae biomass productivities depending on the production technology and efficiency.

It was shown that each assumption during the productivity calculation and area location has a significant influence on the results. Considering the base case productivity of 30,000 $L \cdot ha^{-1} \cdot year^{-1}$ for open pond facilities the cultivation area requirement to achieve the *published targets* would be 9,000 km^2 for the EU, 18,900 km^2 for the US and from 2,300 km^2 for Brazil. For complete replacement of fossil based diesel the land demand increases to 149,300 km^2 for the EU, 106,700 km^2 for the US, 29,700 km^2 for Brazil, and 811,600 km^2 for the world.

The best option to locate the biodiesel facilities would be to concentrate the production locally according to the demand and consequently some sites were proposed that met algae cultivation requirements – favourable climate (small temperature variation) and unoccupied flat land with close proximity to the inputs (water, nutrients and carbon sources). The production would be concentrated in the south of the EU, southeast of US and along the Brazilian coast.

This study also revealed that the improvement of the production technology could significantly reduce the area requirements; photobioreactors have a great potential due to their high productivity. Offshore production also represents a relevant alternative to land requirements and should be considered

especially by the EU which now produces less biodiesel than it consumes and does not have sufficient land available.

Currently, there is no commercial production of third generation biodiesel (only in some pilot plants) and algae biodiesel production is treated as a new field that needs further research, development and investment in order to become economically and environmentally feasible, so presently algae derived fuel is not considered when achieving targets.

It is important to highlight that the objective was not to suggest the total replacement of fossil-diesel by algae biodiesel, but to demonstrate the potential of this source of biodiesel. When considered together with other alternative fuels and diverse feedstocks it is plausible to achieve the targets and even approach the replacement of fossil diesel. This study provided the first analysis at identifying the best places to cultivate algae. It highlights the need for more detailed work in mapping the resources and especially data selection to calculate the productivity more precisely.

Suggestions for further work include the collection of climate data for the productivity period, detailed geo-referenced available area data in order to calculate areas with more accuracy and also the identification of the best species for given climate and locations where nutrients and CO_2 sources are available at a local level. It is also important to continually update the governmental role on regulations and incentives available to the feasibility of the algae technology.

4. Algae Characterisation

4.1. Introduction

Chlorella vulgaris was selected because of the amount of research on this strain, its easy cultivation (more than 1 $mg \cdot mL^{-1}$ after three days) and its availability during this project (already cultivated at the University of Birmingham).

The algae used in the research were from two different sources. The first was purchased from Cranfield University where it was cultivated using f/2 media (Guillard and Ryther, 1962) and stored at - 4°C after freeze drying. The second was cultivated at the University of Birmingham using Bold Basil Media (Nichols and Bold, 1965) and was used direct after three days cultivation (Figure 4.1) or after centrifuging and drying (the growth conditions of the fresh algae are presented in Appendix 2). They will be referred to in this document as f/2Algae and BBMAlgae, respectively.

Figure 4.1. *Chlorella vulgaris* after three days of cultivation

The algae cultivated in the University were grown for the purpose of feeding Daphnia (an aquatic crustaceans) and used in conjunction with the Bioscience Department. Because of this, the media and conditions selected for its growth were not to optimise the lipids content (the lipid content was lower than 8% wt. when there is the potential for 50% wt.). The lipids could be optimised through stressed nitrogen environments or with the restriction of other nutrients.

The algae were characterised for carbon, hydrogen, nitrogen, oxygen and phosphorus content. They were also investigated by scanning electron microscope (STEM) and thermogravimetric analyser (TGA). Furthermore, the lipids, protein content, carbohydrates and high heat value (HHV) content were quantified and the best disruption methods were studied.

The characterisation was completed with the help of Lydia Gurley and the Masters students Matthew Keith, Zoe Preece and Yumna Islam.

4.2. Materials and methods

4.2.1. Cultivation

The BBMAlgae were cultivated in 2.5 L bottles with the addition of 10 mL of growth algae to 1.5 L of fresh media. Air was pumped continuously into the culture for its circulation, light was provided continuously by artificial illumination (two 1.5 m, 58 W triphosphor fluorescent tube lamps) and the room temperature was maintained through air condition at 24°C.

The growth of BBMAlgae was measured by collecting 1 mL sample every 24 hours during the cultivation of three different batches of algae. From this sample, the optical density, the number of cells and the dry mass were measured.

4.2.1.1. Optical density

The optical density was measured by absorbance in a spectrophotometer (Cecil 7500) at 750 nm wavelength. 1 mL of sample was used for each measurement and each sample was tested three times.

The Cecil 75000 is a double beam spectrophotometer equipped with two silicon diode detectors that works in a wavelength range of 190 to 1,100 nm (Cecil, 2016). The light beam passes through the sample (inside a cuvette) and the absorbance is measured from the intensity of light transmitted.

4.2.1.2. Number of cells

The number of cells in suspension per mL was counted using a hemocytomer and an Olympus BX50 microscope. The hemocytomer is an instrument designed for visual counting cells; it is a thick microscope slide (coverslip 0.1 mm from the surface) with a square grid comprising nine 1 mm^2 squares, with the central square divided into 25 smaller squares of 0.04 mm^2 (Fey et al., 2007). A representation of the hemocytomer and an image produced from an algae sample can be seen in Figure 4.2. The cells are counted in five of the small squares to give the number of cells in 0.02 µl and this value is multiplied by 50,000 to adjust to the number of cells in 1 mL.

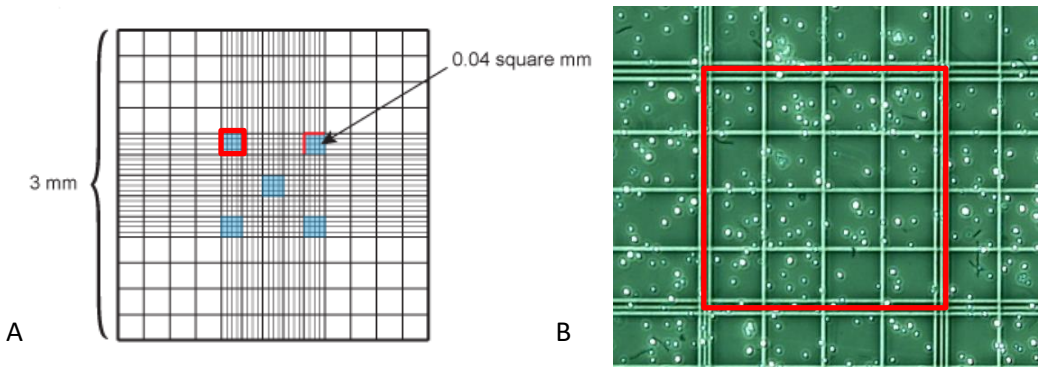

Figure 4.2. A) Hemocytomer representation (Fey et al., 2007) B) Microscope view of the sample zoomed in one square of 0.04 mm²

4.2.1.3. Concentration

The concentration (mass per volume) was measured after drying 1 mL of solution in an Eppendorf tube overnight in a freeze dryer (where the sample is frozen and the water removed by sublimation) until the weight stabilised, giving the mass of solids in the sample.

4.2.2. Harvesting

The culture is harvested after three days when the optical density has reached 0.8. The culture is then centrifuged (J2 Beckman) at 5,000 rpm for 30 minutes at 10°C to achieve the concentration desired for further testing. If dry algae were required, the algae were transferred to the freeze dryer after centrifugation for a minimum of 12 hours until the weight became constant.

4.2.3. Disruption methods

- Control
    - Algae were used directly after harvesting at the desired concentration.

- Manual grinding
    - Dry algae were ground using a ceramic pestle and mortar for approximately 5 minutes until a fine, uniform powder was obtained.
- Chemical treatment
    - The method used to extract proteins was adapted from Safi et al. (2014). The sample pH was adjusted to pH 12 using sodium hydroxide (NaOH), heated to 40°C, stirred for 2 hours and then the supernatant pH was reduced to pH 3 with 0.1 M hydrochloric acid (HCl) in order to precipitate the proteins (which were quantified by Lowry Method described in section 4.2.8).
- Microwave
    - A Hinari Microwave (Model MX707TC, 800 W, 2.45 GHz) was used at full power in 10 s pulses (10 s on: 10 s off) for 10 min to disrupt the samples.
- Ultrasonic bath
    - Langford Sonomatic® Ultrasonic Bath (S1400, 1,050 W, 50-60 Hz) was used without heating the samples for at least 30 min.
- Ultrasonicator (Us)
    - An ultrasound probe (Sonics Vibracell Model CV18 – 50/60 Hz) was used with amplitude from 45.6 to 91.2 µ (total energy supply set from 2,500 J to 5,000 J). This method was also adapted from Safi et al. (2014).

4.2.4. Compositional analysis – (CHNOP)

Compositional analysis was carried out to quantify the mass fractions of carbon, hydrogen, nitrogen, oxygen and phosphorus (CHNOP).

The CHNO quantification was carried out by Medac Ltd. (Surrey, UK) where elemental analysis was measured by a combustion method. Three samples of BBMAlgae representing three different batches of cultivation and one sample of f/2Algae, as there was only one batch of this biomass available, were sent to Medac where the samples were analysed in duplicate.

Phosphorus was quantified by the Molybdate Reactive Phosphorus Method (MRP-Method) (Menzel and Corwin, 1965). The dry algae sample (around 2 mg) was dissolved in water (10 mL) with the addition of potassium persulphate solution (5% vol., 2 mL) and it was autoclaved for 60 minutes at 120°C. With that, there is the oxidative hydrolysis of the organically bound phosphorus to orthophosphate. After the autoclave time and the cooling of the sample, a reagent mixture (2 mL), according to the table below, was added and then the absorbance was read at 882 nm after 30 min.

Table 4.1. Reagent mixture

Solution	Quantity (volume)
Sulphuric Acid (2.5M)	50%
Ammonium Molybdate (4% vol.)	15%
Potassium Tartare (1mg SB·mL^{-1})	5%
Ascorbic Acid (0.1M)	30%

Blanks (samples with only distilled water) were made alongside the samples and phosphate calibration (with monopotassium phosphate – KH_2PO_4 at a concentration of 1,000 µg PO_4-P·mL^{-1}) was conducted in order to calculate the total phosphorus using Equation 4.1 below. Each sample was analysed in triplicate.

$$Total\ P\ (\mu g\ P \cdot l^{-1}) = \frac{C_{cal} * V_{cal} * (A_S - A_{Blank})}{V_S * (A_{cal} - A_{Blank})} \quad \textbf{Equation 4.1}$$

Where C_{cal}, V_{cal} and A_{cal} are the concentration (µg P·L^{-1}), volume (mL) and absorbance of the calibration solution, respectively. V_s and A_s are the volume (mL) and absorbance of the sample, respectively and A_{Blank} is the absorbance of the blank sample.

4.2.5. High heating value

The high heating value (HHV) was calculated from the elemental composition following the model used in the literature (Clarens et al., 2010) and presented in Equation 4.2.

$$HHV\ (kJ/kg) = 35160\ C + 116225\ H - 11090\ O + 6280\ N \quad \textbf{Equation 4.2}$$

Where C, H, O and N are the weight fraction of carbon, hydrogen, oxygen and nitrogen, respectively, in the biomass. This model also agreed with others from the literature (Friedl et al., 2005, Sudhakar and Premalatha, 2015).

4.2.6. SEM

For imaging the biomass, a FEI/Philips XL30 FEG ESEM – Field-Emission Gun Environmental Scanning Electron Microscope – was used, at a voltage of 10 kV and resolution of 1.5 nm.

The samples were prepared in order to compare the source of biomass, the available drying methods and techniques for cell disruption. The samples were dried and then coated with platinum before imaging.

4.2.7. Thermogravimetric analysis – TGA

A thermogravimetric analyser (TGA) was used to quantify the amount of moisture, volatiles and minerals in the samples. The analysis was conducted with dried algae (around 3 mg stored at -20°C). The biodiesel process works between 250°C and 300°C, so the TGA was set up in a nitrogen atmosphere for an inert environment, and the temperature increased from 25°C to 500°C at a rate of $10°C \cdot min^{-1}$. A similar analysis was done by Reddy et al. (2014) and Peng et al. (2001).

In conjunction to the TGA, the amount of ash was determined following a procedure from the literature (Sluiter et al., 2005), where the biomass is burned in air in a muffle furnace using a ramp rate of $5°C \cdot min^{-1}$. The analyser starts at room temperature and increases up to 105°C and is held for at this temperature for 12 minutes, increased to 250°C and held for 30 minutes and then increased to 575°C and held for 180 minutes. The remaining mass is the ash content. This procedure was repeated in triplicate.

4.2.8. Proteins

The protein content was estimated using two different methods. The first was by direct conversion from the nitrogen content using a conversion factor of 5.47. This conversion factor was taken from the literature based on the average protein molar mass and nitrogen in the biomass (Ursu et al., 2014). The second method was treatment with lysis solution (LS) followed by a disruption method, sodium dodecyl sulphate (SDS) solution addition to help the protein precipitation (González López et al., 2010) and quantification by the Modified Lowry Method (Lowry et al., 1951).

The lysis solution was prepared as described by González López et al. (2010) from 5 mL Triton X-100, 372.2 mg of EDTA (ethylene diamine tetra acetic acid disodium salt), 34.8 mg of phenyl methyl sulphonyl fluoride, and made up to 1 L using ultrapure Milli-Q water. The SDS Solution was prepared at a concentration of 0.05 $g \cdot L^{-1}$. The methodology is summarised in Figure 4.3.

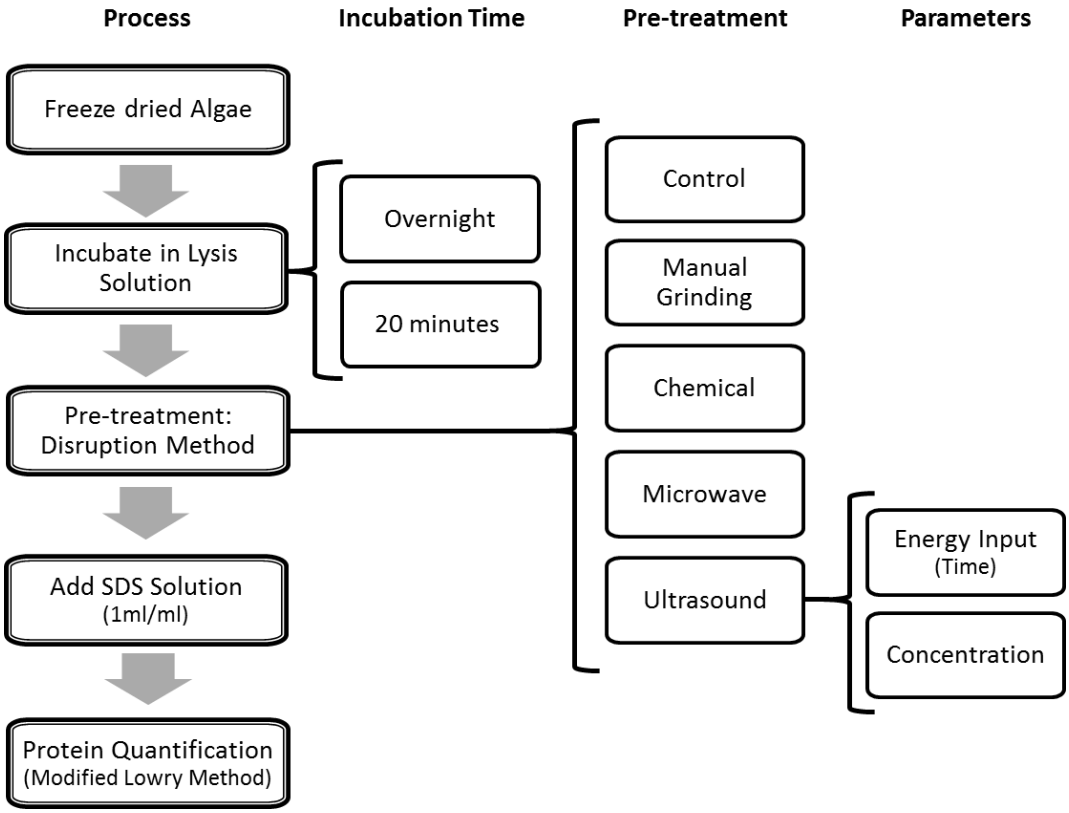

Figure 4.3. Protein determination methodology

A Modified Lowry Protein Assay Kit from Thermo Scientific was used to determine the protein amount. This method uses colorimetric techniques to determine protein content through the reaction of copper with peptide bonds (Lowry et al., 1951). Bovine Serum Albumin Protein (BSA) was used to create calibration curves (BSA concentrations from 0 $mg \cdot L^{-1}$ to 1,500 $mg \cdot L^{-1}$) with R^2 values ranging from 0.965 to 0.998 each time the Lowry method was used. A calibration curve example is presented in Figure 4.4.

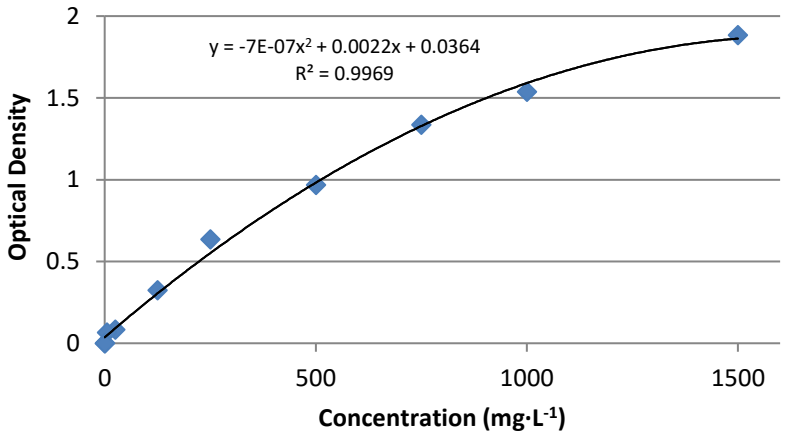

Figure 4.4. Example of a calibration curve in the spectrophotometer with BSA

The protein analysis was conducted on 0.2 mL aliquots (0.1 mL of sample and 0.1 mL of SDS solution). The absorbance was measured using a Cecil CE7500 ultraviolet spectrophotometer at a wavelength of 750 nm. The yield was calculated by the mass of protein per mass of algae.

The effect of incubation time on protein extraction was investigated by incubating the samples (100 mg of algae mixed with 8 mL of LS) at 4°C, either overnight or for 20 minutes before the disruption treatment was started.

The effect of algae concentration in protein extraction was investigated varying the algae mass (25 mg, 50 mg, 75 mg or 100 mg) diluted in 15 mL of LS and treated using the ultrasound probe at 40% amplitude, for pulses of 5 s on, 10 s off for 30 minutes (around 2,500 J in total).

In order to explore the energy for optimum protein yield, the time exposure in the ultrasound probe was varied between 10 min and 75 min, as this would change the total energy input to the sample. The pulse time (5 s on and 10

s off) and probe depth (1.5 cm) in the sample remained constant throughout the experiments. The temperature of the solution was also monitored throughout the experiments, with readings taken every 15 min (or every 5 min of ultrasound exposure) with no detectable variation.

Both techniques (nitrogen conversion and LS with Lowry method) were compared in order to evaluate the use of the elemental analysis for protein estimation.

4.2.9. Lipids

The lipids were quantified using soxhlet and vacuum filtration extraction with different pre-treatments (control, manual grinding, ultrasound bath, and ultrasound probe) and solvents (chloroform and methanol – 2:1, 1:1 and 1:2 v/v, hexane, CO_2). Confirmation of the values were also done with the algae directly transesterified to FAEE and FAME in the presence of a catalyst (bench reaction).

The oil was extracted using the soxhlet method. The soxhlet is a semi continuous method to extract substances with low solubility in solvents, and the apparatus is showed in Figure 4.5. The biomass is inserted in a cellulose filter paper in the extraction chamber where it is washed by the solvent (around 10 times over a period of approximately 4 hours) in a system where the solvent is heated in the boiling flask, distilled and then returned to the sample until it is periodically siphoned off back to the flask, taking non-volatile dissolved compounds with it. At the end of the extraction the collected solvent was evaporated in a rotary evaporator using heating blocks (heating the samples to

around 80°C in an inert environment with the use of nitrogen gas for the complete evaporation of the solvent). The extract (lipids) is the remaining solid phase. The solid lipid extracted using the soxhlet apparatus was then converted to methyl esters or ethyl esters as described in the section below.

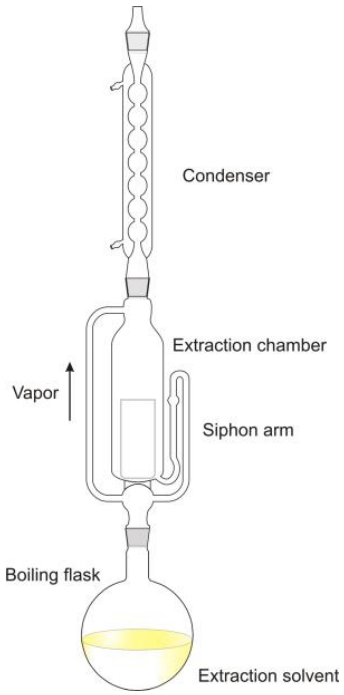

Figure 4.5. Soxhlet apparatus (Generalic, 2015)

The supercritical CO_2 extraction of lipids was done in a 300 mL Parr bench top batch reactor 5500 made from 316 stainless steel, equipped with a variable speed magnetic drive stirrer and external electrical heater set at 50°C and with the pressure exceeding 90 bar. The algae sample with no pre-treatment (around 40 mg) was inserted in the reactor which was then pressurised with CO_2 to 50 bars and the temperature was raised to 50°C. After 1 hour the reactor was depressurised and the sample was removed for FAME conversion.

4.2.9.1. Conversion

To convert to FAME, the extracted lipid was dissolved in 1 mL hexane and transferred to a 5 mL micro reaction vessel to which 100 µl of 2,2-dimethoxy propane and 2 mL of hydrogen chloride – methanol (1.25 M HCl) was added. While to convert to FAEE, the extracted lipid was dissolved in an ethanol and catalyst mixture (1:0.05 w/w).

The mixture was heated to 85°C in a heating block and held at that temperature for 30 minutes before allowing it to cool. 1 mL of water and 1 mL of hexane were then added to separate the polar and nonpolar components in to two layers; the top layer (hexane plus nonpolar, i.e. FAEE) was transferred to an Eppendorf tube with anhydrous sodium sulphate to remove any remaining water. A sample of this hexane phase (0.5 mL) was transferred into a gas chromatography (GC) vial containing 0.5 mL of an internal standard (heptadecanoate methyl ester dissolved in hexane at 40 $\mu g \cdot mL^{-1}$) for analysis by the GC.

4.2.9.2. Bench reaction

In order to verify the values obtained by soxhlet, bench reactions in the presence of a catalyst were performed. Sulphuric acid (100% wt.), acetyl chloride, zinc aluminate and sodium hydroxide were used in concentrations of 5% w/v to ethanol. These catalysts were chosen because were the most used in the literature and because of its availability. The sodium hydroxide is the standard catalyst used with other biomass oils; sulphuric acid and acetyl chloride were

suggested by the literature to deal with high amount of FFA and algae biomass (Laurens et al., 2012) and the zinc aluminate is a new catalyst provided by the Federal University of Bahia because of a partnership between the universities .

The algae (between 10 to 40 mg) were added to the ethanol catalyst mixture (2 mL), exposed to the ultrasonic bath for 30 minutes and then to heat (using a heat block) at 85°C for one hour. After cooling, 1 mL of hexane and 1 mL of water were added to force the separation as above and a sample of the hexane layer was analysed in the GC as before. The GC methodology is described in section 4.2.9.3.

4.2.9.3. Gas chromatography and mass spectrometry

Gas chromatography (GC) was used to quantify the FAME and FAEE in the samples. The sample solution, diluted in a solvent (in this case hexane), is injected into the GC and is transported by the carrier gas to a column where its constituents are separated due to interactions with the stationary phase of the column. When the components leave the column (each one at a different time) they are identified by a flame ionization detector (FID) and quantified using a relationship between the areas of the components and a known standard sample (heptadecanoate methyl ester) (Shimadzu, 2016). The GC output is given in a graph of signal magnitude by retention time and the area under the curve can be related to the mass fraction. Figure 4.6 shows a GC representation.

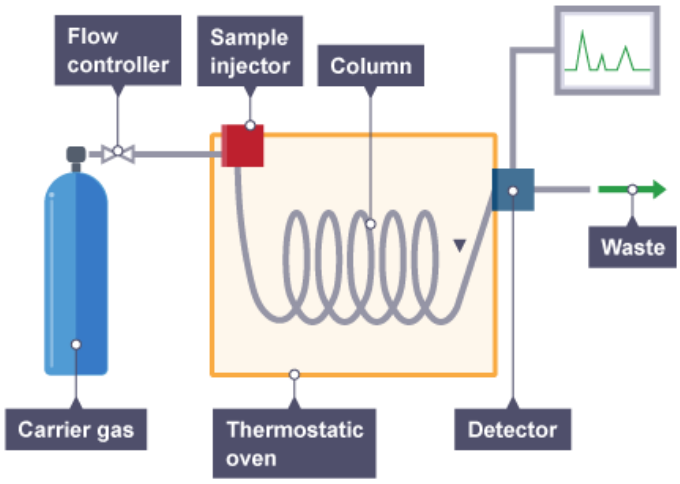

Figure 4.6. Gas chromatography scheme (BBC, 2014)

Firstly, to identify the sample's components, a GC with a mass spectrometry (MS) connected (known as GC-MS) was used. The GC-MS is able to separate the chemicals (ionizing them) and identify them by their mass spectrum which can be correlated with a database. After this identification, it was possible to buy the appropriate standards for the components present and make the calibration curves to use the GC with all samples. The calibration curves from the GC for FAME and FAEE are presented in Appendix 3.

FAME analysis was performed in a GC-2010 Plus Shimadzu GC with ZB-5 capillary column (30 m length, 0.53 mm diameter and 1.50 µm film thickness) and FID at 300°C. The oven was set up to start at 100°C, heating to 190°C over 30 min, holding for 10 min and then increasing to 300°C over 20 min.

FAEE was quantified using a 6850 Agilent Technologies gas chromatographer with a DB-5HT capillary column (15 m length, 0.32 mm diameter and 0.1 µm film thickness) and FID at 325°C. The oven temperature was set to 100°C and raised at a rate of 30°C·min^{-1} to 190°C where it was held for 10

minutes, before a further ramp of 5.5°C·min^{-1} to 300°C where it was held for 15 minutes. Helium was employed as the carrier gas at a flow of 2 mL·min^{-1} and nitrogen as the make-up gas with a splitless injection (300°C).

4.2.10. Carbohydrates

The Carbohydrates were calculated by difference considering that the biomass is formed of carbohydrates, proteins, lipids and minerals, so its value can be found by using Equation 4.3:

$$\%_{\text{carbohydrate}} = 100 - \left(\%_{moisture} + \%_{protein} + \%_{lipid} + \%_{mineral}\right) \quad \textbf{Equation 4.3}$$

4.3. Results and discussion

4.3.1. Cultivation

Figure 4.7 shows the growth curve in the first four days based on three different batches.

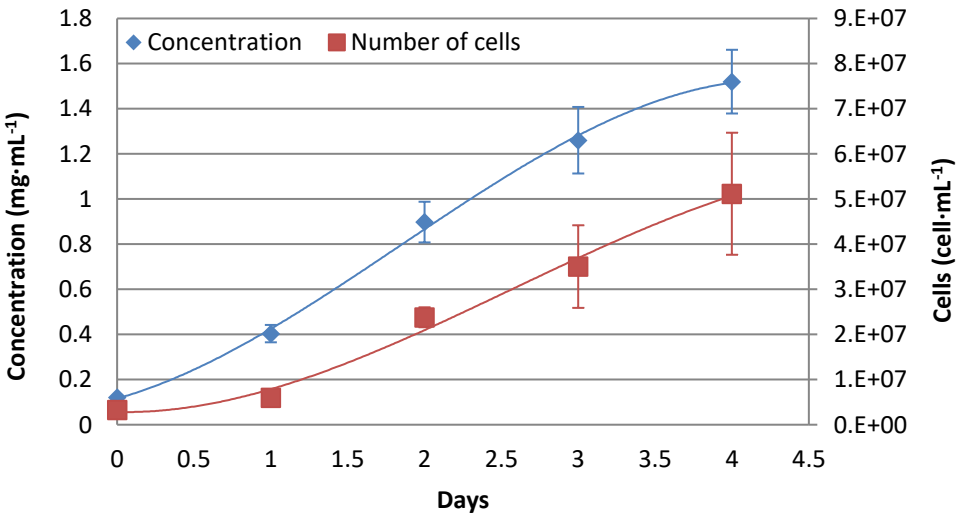

Figure 4.7. Growth curve for four days (mass concentration and cell amount)

It is possible to observe from the error bars that the concentration after this period is similar for the different batches so it is acceptable to use them for further experiments. After the fourth day the growth decreases, hence it was decided to harvest before this happens.

Figure 4.8 demonstrates that the optical density can be used as a parameter of concentration in order to harvesting, since it has a linear relationship with algae concentration (R^2=0.97) and cells number (R^2=0.94).

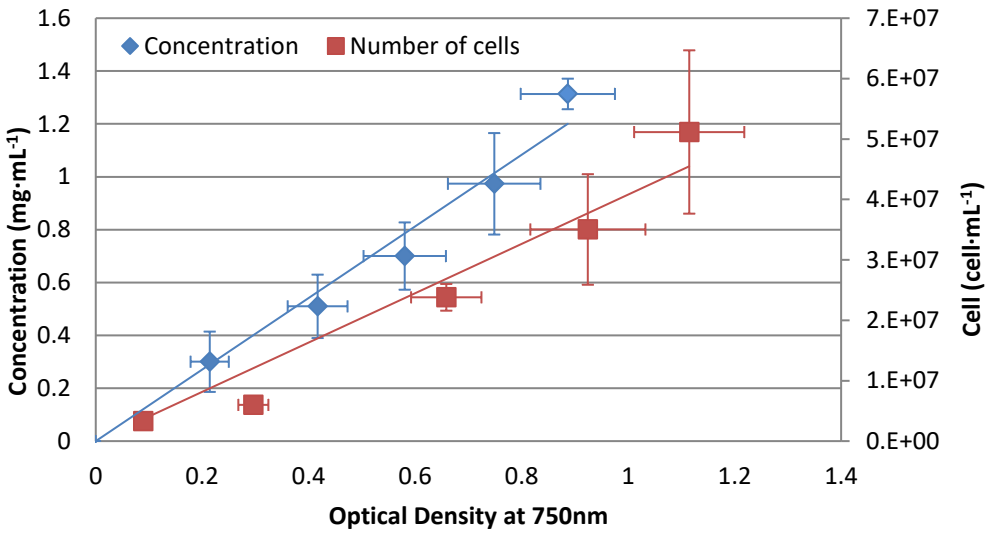

Figure 4.8. Optical density relationship with algae produced (mass concentration and cell amount)

The algae were harvested after three days when the optical density was greater than 0.8 (and therefore a mass concentration of around 1 $mg \cdot mL^{-1}$ as presented above).

During the cultivation growth analysis, it was possible to understand the relationship between the cell number, weight and optical density. This is important for the speed of the harvesting and concentration decisions after this

stage, since the optical density is a quick parameter to measure and only requires 1 mL of sample.

4.3.2. Comparing the algae source

The growth conditions of the algae make differences in the algae characteristics and composition. The main results of algae elemental characterisation are presented in the Table 4.2 below:

Table 4.2. Algae elemental analysis and HHV

Compound (wt.)		f/2 Media	BBM Media
Carbon	%	36.58 ± 0.13	47.24 ± 1.85
Hydrogen	%	5.66 ± 0.02	7.04 ± 0.16
Nitrogen	%	2.65 ± 0.1	8.01 ± 0.76
Oxygen	%	36.43 ± 0.13	30.19 ± 0.18
Phosphorus	%	0.037 ± 0.002	0.126 ± 0.068
Others[a]	%	18.64	7.39
HHV[b]	$MJ \cdot kg^{-1}$	15.57	21.95

[a] Others = 100 – (C + H + N + O + P)
[b] Calculated by Equation 4.2

The main element in algae is carbon. It represents 36.6% wt. in f/2 media and 47.2% wt. in BBM media, followed by oxygen which represents 36.4% and 30.2% wt., respectively. The high nitrogen in the BBMAlgae (8% wt. compare to 2.65% wt. for f/Algae) indicates high protein content which is an advantage compared to other biomass feedstocks which usually contains less than 1% wt. nitrogen (Sudhakar and Premalatha, 2015).

The standard deviation for the BBMAlgae shows that even with the same media and the same cultivation time, it is not possible to obtain the same characteristics in all batches. Due to the controlled indoor cultivation, these

differences can be ignored and algae from different batches were used. It is acceptable that in the scale-up process variations in composition will occur, especially because of the weather.

The high amount of others in the f/2Algae indicates the presence of unexpected compounds. The SEM images from both sources (Figure 4.9) presented below can confirm that the f/2Algae developed a contamination with *Pennales diatoms*, a heterokont algae with a silica cell wall with a linear oval shape (Barron, 2003). This contamination was also revealed later by the GC-MS which identified a large amount of silica compounds besides the oils.

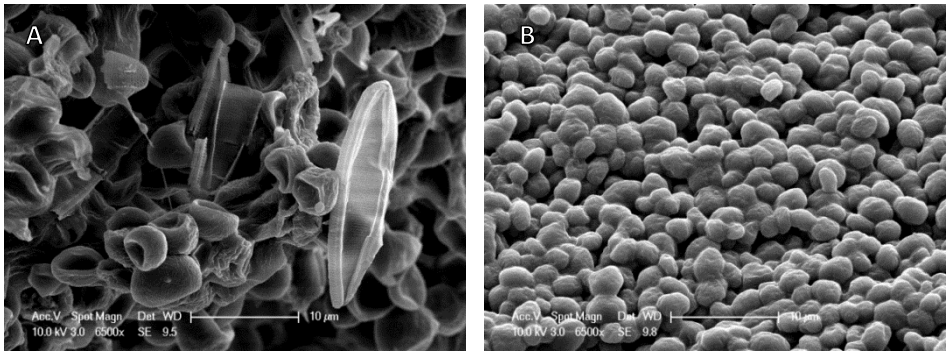

Figure 4.9. SEM images – Cultivation media A) f/2Algae B) BBMAlgae

Other important differences between the samples are the concentration of cells and the cell conditions. The BBMAlgae are more concentrated and have more defined circular cell walls. One explanation for this can be the age of the material, since the f/2Algae has been purchased frozen and the BBMAlgae is collected fresh, which does not allow time for decomposition.

The high heating values from both algae agrees with the literature which indicates values from 15 to 25 $MJ \cdot kg^{-1}$ (Kebelmann et al., 2013, Clarens et al.,

2010). It also indicates a better potential for fuel production of the BBMAlgae which has an HHV 70% larger than the f/2Algae.

Because of the identification of these variables, such as the silica contamination and lower HHV, the f/2Algae was only used for methodology tests and no longer used for further experimentations.

4.3.3. SEM Analysis of pre-treatments

The first pre-treatment tested was the way the algae were dried. The drying method interferes with the cell wall behaviour; the use of the freeze dryer also removes the water from inside the cell while the use of temperature (drying cabinet) maintains the spherical form of the cells. In light of this, the freeze dryer helps in the wall disruption. SEM images are presented in Figure 4.10 which illustrates this phenomenon.

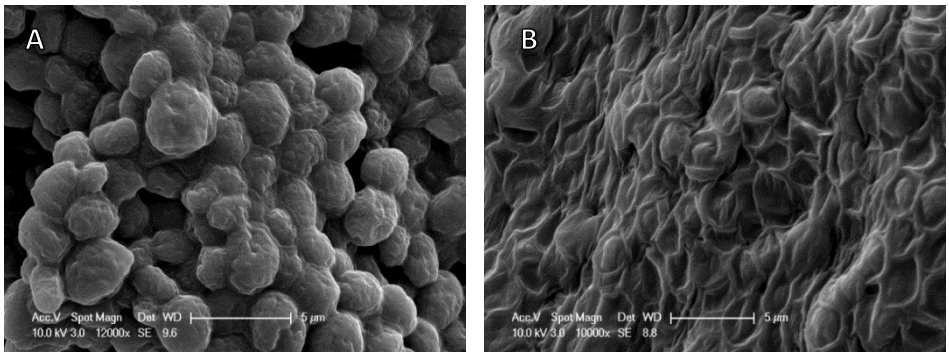

Figure 4.10. SEM images – Dry method A) Dried in drying cabinet B) Dried in freeze dryer

The freeze dried algae was used for the characterisation profile: TGA analysis, lipids and protein contents.

Both drying methods were also tested in combination with the cell disruption method and then analysed by SEM (Figure 4.11). In all cases, the cells

were better disrupted after the freeze dryer as suggested before by the obtained SEM images. The ultrasonicator proved to be the most efficient method to disrupt the cells, followed by the ultrasound bath and the manual grinding. Figure 4.11 contrasts these disruptions, where it is possible to see some remaining cells after the manual grinding but not after the cells have been submitted to the freeze dryer or ultrasonicator.

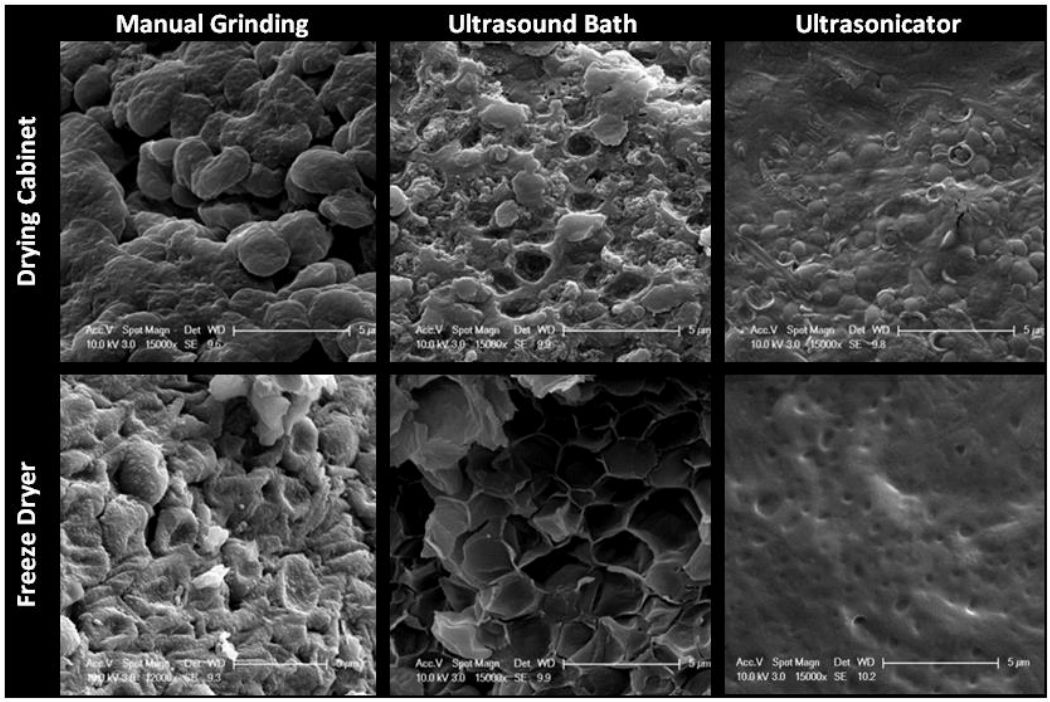

Figure 4.11. Comparison between pre-treatments

4.3.4. Composition and thermal analysis

The algae composition can be expressed as moisture, proteins, lipids, carbohydrates and ash. The analysis of this composition started with a TGA test where it was possible to check the moisture and the volatiles compounds. Then ash, proteins and lipids were quantified, and finally the carbohydrates were calculated from the difference. The TGA results are shown in the Figure 4.12.

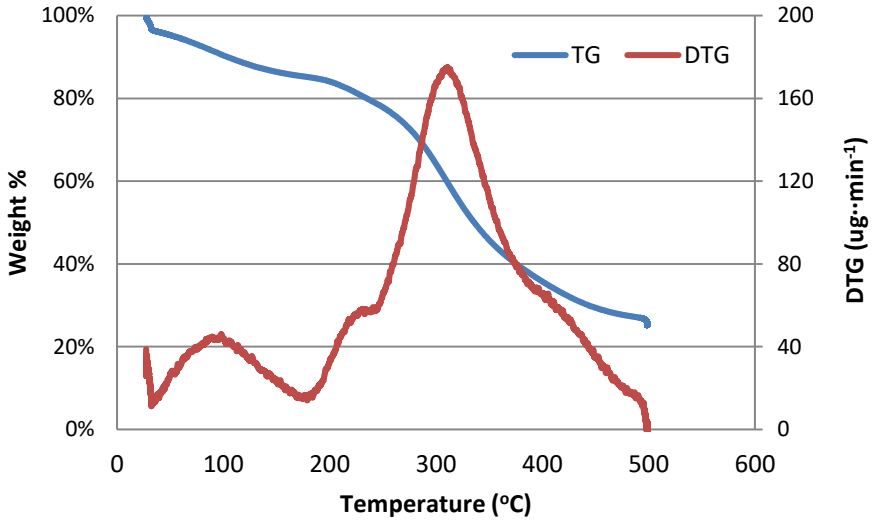

Figure 4.12. TGA – Freeze dried BBMAlgae

The TGA illustrates that the largest weight loss occurs between 200°C and 350°C, where the volatile compounds present in the algae are lost. The first stage, up to 140°C, is the loss of moisture (15% wt.) and is from the remaining water in the cells after the freeze drying process. The mass remaining (25% wt.) after 500°C is char formed by the fixed carbon and ash. The mass of ash was determined from the gravimetric analysis after 575°C in air in the muffle furnace. The average ash mass % was 10.22% ± 0.29%. The volatiles are lost mainly from 250°C to 400°C which represents the decomposition of hydrocarbon chains of fatty acid (Kebelmann et al., 2013). Table 4.3 summarises the main composition obtained by TGA.

Table 4.3. Compounds Identified by thermogravimetric analysis

Compound	Mass %
Moisture	15
Volatile matter	60
Fixed carbon	15
Ash	10

It is important to understand that the volatile compounds start to be lost at 200°C, which is a lower temperature than the reactor operates at, so some mass may be lost during the process of direct transesterification tested in the next chapter.

4.3.4.1. Proteins

The protein in the algae estimated by the nitrogen content was 48.6% wt. (from a nitrogen amount of 8.96% wt.). This value agrees with the literature where *C. vulgaris* has been shown to contain a protein weight between 30% and 55% depending on the growing conditions (González López et al., 2010).

As demonstrated before, the ultrasonicator was the best disruption method. A comparison of the effectiveness of the various methods on protein release in frozen algae is presented in Figure 4.13.

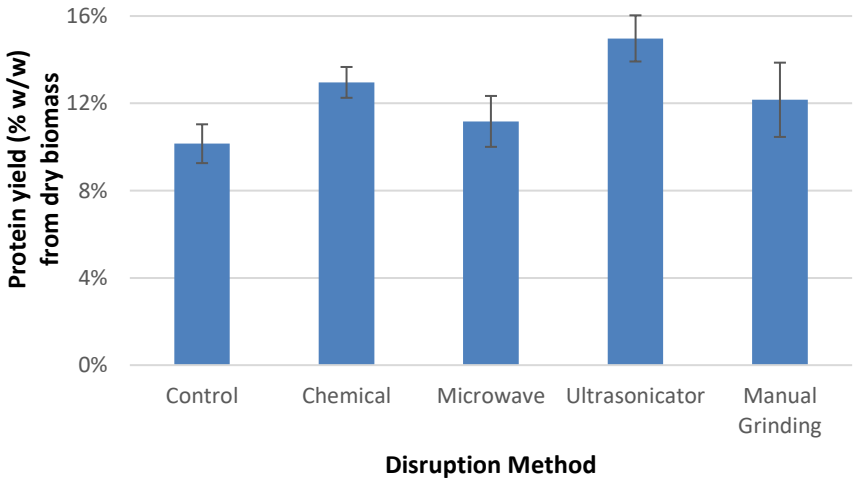

Figure 4.13. Comparison of protein yield from different cell disruption methods

The control, the algae only treated with LS and SDS, achieved 10.1% ± 0.89% wt. which means that with no disruption method applied there is a spontaneous protein leak.

Microwaving and manually grinding did not bring a statistically significant improvement in protein extraction (11.2% ± 1.16% and 12.2% ± 1.87%, wt. respectively). Although the microwave consumed energy of 4,800 $MJ \cdot kg^{-1}$ of algae, much of this energy was dissipated to the environment. Another reason for the low protein yield could be the effect of microwaves on protein structure; studies have shown that even non-thermal effects cause degradation of protein structures (Porcelli et al., 1997). The SEM imaging presented indicates that cells were not really broken after the manual disruption method, which might explain negligible effect.

Utilising a chemical solvent, heating to 40°C and stirring for 2 hours was reasonably effective, giving a yield of 13.0% ± 0.70% wt. (an increase of almost 30%). Alkali conditions may accelerate cell lysis and lower the energy requirement; however it appears that high pH alone is insufficient to achieve complete disruption as suggested by Ursu et al. (2014) who also used a high pressure cell disruptor to release proteins.

The highest protein content was obtained when using ultrasound to disrupt cells with a yield of 15.0% ± 1.06% wt. after 37.0 ± 2.48 $MJ \cdot kg^{-1}$ of energy had been applied, and consequently this method was further analysed.

The effect of lysis incubation time was tested for its effect on the efficiency of ultrasound for different energy inputs and the results are presented in Figure 4.14.

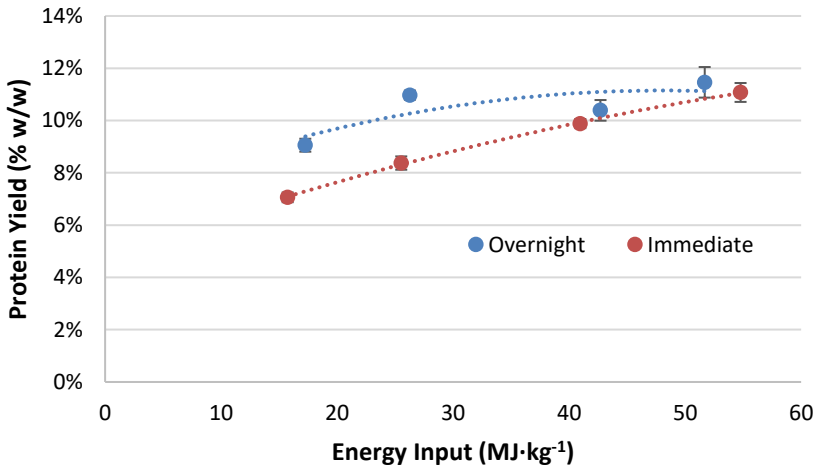

Figure 4.14. Protein yield obtained by ultrasonic disruption when 1) left in lysis solution overnight and 2) when immediately added (20 minutes wait)

The protein yield when the algae were left to imbibe in solution overnight was higher than when lysis was added on the same day. However, as the energy approaches 54 MJ·kg^{-1} of initial biomass, the results converge. This indicates that extending the time spent in the lysis solution weakens the cell membrane, but as energy input increases, this effect is compensated and becomes irrelevant. This is expected as the effect of lysis solution alone is not as significant as the effect of ultrasound, as shown by the control sample in Figure 4.13. There is no additional advantage to soaking *C. vulgaris* in a lysis solution before disruption at high energy inputs (more than 55 kJ·g^{-1}), so the next experiments with higher energy exposure were done immediately after adding the lysis. This has important economic

impacts when considering an industrial scale process; by introducing ultrasonic waves immediately, processing time will be minimised.

The effect of dry cell weight (DCW) on protein yield was investigated under the same conditions (incubation in lysis solution for 20 minutes and Us with pulses of 5 s on and 10 s off, for 30 min). The highest protein yield was achieved using a DCW concentration of 3.33% w/v; this and the other results are shown in Figure 4.15. The concentration is important for the disruption mechanism due the interaction between the cells and solvent, during the ultrasonic extraction a cavitation effect occurs, and in dilute solutions there are not enough cells for the collisions and in high concentrate samples the Us has difficult to propagate the media (Mercer and Armenta, 2011, Günerken et al., 2015).

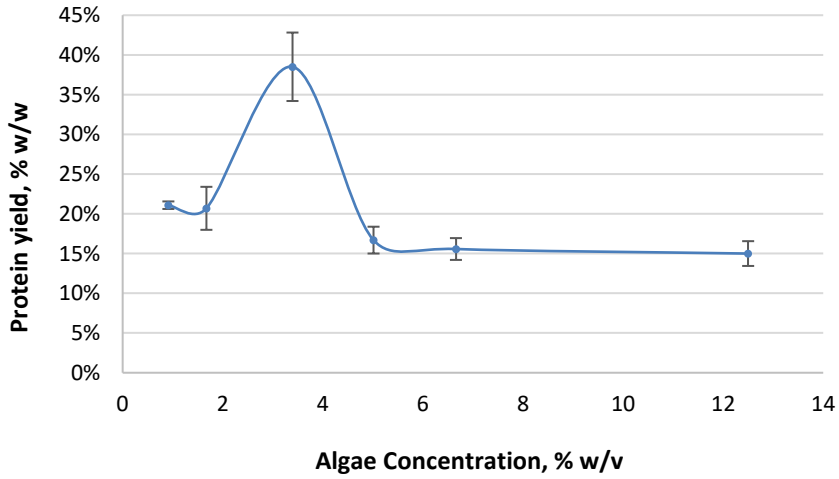

Figure 4.15. Effect of changing DCW concentration for a similar ultrasound energy input (2500 J with pulses of 5 seconds on and 10 seconds off)

After finding the best incubation time and concentration, the Us was used for longer durations to increase the energy input. As expected, the level of disruption, and hence protein yield, increased with a greater energy input as

presented in Figure 4.16. This continues until total cell lysis was achieved with 210 MJ·kg^{-1} supplied to the sample. It is assumed, that at this point, cell walls were entirely broken down and all protein was suspended in solution, therefore, supplying more energy did not result in a greater protein yield. Above 210 MJ·kg^{-1} there is a reduction in protein content, possibly due to protein degradation by ultrasound (Borthwick et al., 2005). Further work should be done to identify the protein form and its denaturation.

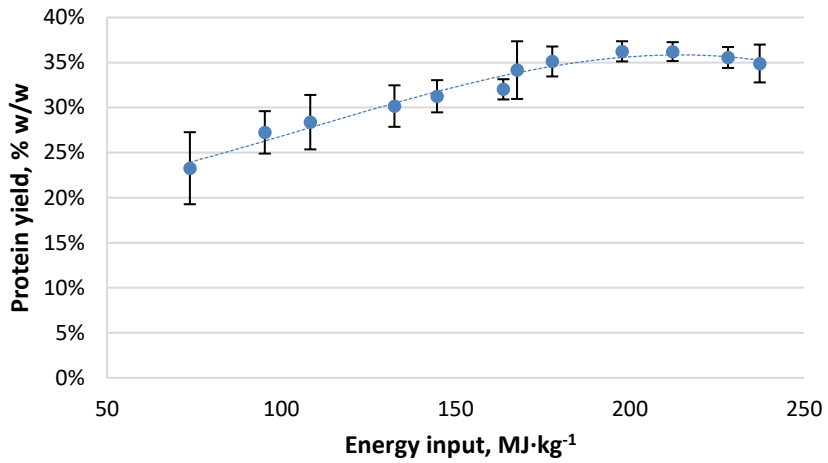

Figure 4.16. Effect of increasing ultrasound energy input (by increasing treatment time) on protein yield

The maximum protein extracted was 36.24% ± 1.16% wt. after 60 minutes. It was obtained from dried algae incubated for 20 minutes in LS with a concentration of 3.4 mg·mL^{-1} and energy supply of 210 MJ.

This value gives a nitrogen factor of 4.02 (calculated by the nitrogen fraction weight and protein amount) which is lower than the other factors found in the literature (Ursu et al., 2014, Safi et al., 2014), which means a larger presence of other components with nitrogen. Calculating protein content through

elemental analysis has the potential to overestimate protein content due to the presence of other nitrogen containing compounds (González López et al., 2010). It is acceptable therefore to obtain 36% wt. as the maximum amount of protein, since this specific culture was not prepared to maximise protein content, as the protein content in *C. vulgaris* can achieve up to 55% (González López et al., 2010).

4.3.4.2. Lipids

The lipids were extracted with the use of different solvents and techniques. The first samples (extracted by hexane) were analysed in the GC-MS to identify the composition of FAEE and FAME. The main five components that were identified in their fatty acid (FA) and FAEE form are presented in Table 4.4. Knowing this profile, it was possible to buy pure standards, whose calibration curves are presented in the Appendix 3.

Table 4.4. FA and FAEE identified in the GC-MS

Fatty Acid (FA)		**Fatty Acid Ethyl Ester (FAEE)**	
$C_{16}H_{32}O_2$	Palmitic Acid	$C_{18}H_{36}O_2$	Ethyl Palmitate
$C_{18}H_{36}O_2$	Stearic Acid	$C_{20}H_{40}O_2$	Ethyl Stearate
$C_{18}H_{34}O_2$	Oleic Acid	$C_{20}H_{38}O_2$	Ethyl Oleate
$C_{18}H_{32}O_2$	Linoleic Acid	$C_{20}H_{36}O_2$	Ethyl Linoleate
$C_{18}H_{30}O_2$	Linolenic Acid	$C_{20}H_{34}O_2$	Ethyl Linolenate

This composition is in agreement with the literature for the lipids profile of the *Chlorella vulgaris* (Dejoye et al., 2011). The total FAME and FAEE were quantified by the sum of the individual components.

The solvents' capacity for extraction can be influenced by polarity and its capacity of cell penetration. In this study the following solvents were tested:

hexane, the most traditional lipids solvent in the literature; methanol:chloroform combined in volume ratios of 2:1 and 1:2, based on the method presented by Bligh and Dyer (1959); and also the use of supercritical CO_2.

Total lipid content could be assumed as the total mass extracted by the soxhlet (Sudhakar and Premalatha, 2015), but in the algae case, this value is overestimated since other compounds, such proteins and pigments, are also extracted (Laurens et al., 2012). This especially occurs when chloroform is used, where the extract is observed to have a strong green colour because of chlorophyll pigments. Thus, the total lipid was considered as the total FAME produced after transesterification, as described in 4.2.9.1.

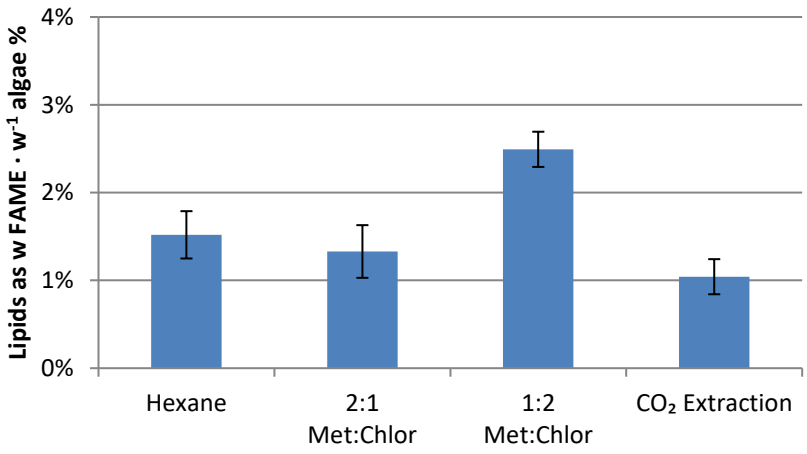

Figure 4.17. Lipid as FAME by different solvent extractions

As shown in Figure 4.17, the use of 1:2 methanol:chloroform gave the highest extraction rate after conversion to FAME. It can be explained by the addition of a polar co-solvent (methanol) that is able to break the lipid-protein interactions present in the biomass (polar lipids, such as phospholipids and

cholesterols, are usually bound to the proteins in the cell walls by hydrogen bonds) (Halim et al., 2011).

On the other hand, supercritical CO_2 was not as efficient. The literature suggests that supercritical CO_2 would have chemical and physical properties that are able to facilitate the cellular permeation (Halim et al., 2012), but results have shown that extraction would be similar to that of hexane (Mendes et al., 2003), with the advantages that there would be no solvent residues in the end, the process would be faster (less than 30 minutes compared to at least 4 hours) and that the quality of the extract would be better (the lipids from CO_2 extraction are mainly linolenic while those from hexane are palmitic and oleic). Consequently, specific research to improve this method has been reported (Dejoye et al., 2011).

Based on the best solvent, the soxhlet extraction was performed after different disruption methods (Figure 4.18) in order to check its improvements for the lipids extraction (Halim et al., 2012).

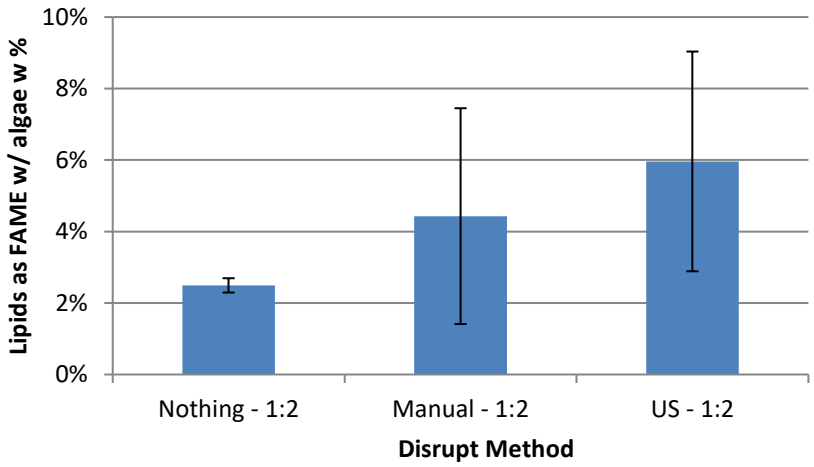

Figure 4.18. Lipids % after different pre-treatments

The potential of disruption methods were proven once more with the lipid extractions. This was also shown before by several studies (Araujo et al., 2013). Here we observed that Us improved the amount extracted by a factor of around 2.5, achieving an average of 6.0% wt. The high standard deviation of the disruption methods may be due to the differences in energy applied in each reproduction since this can be modified with the operator, sample size and concentration. In the best scenario, using the ultrasonicator to disrupt the cells and 1:2 methanol:chloroform as solvent, 6.0% w/w lipids as FAME was obtained.

In order to check the standard method to produce biodiesel, direct transesterifications to FAEE were performed using four different catalysts, following the method described in 4.2.9.2 with dry algae. The results are displayed in Figure 4.19.

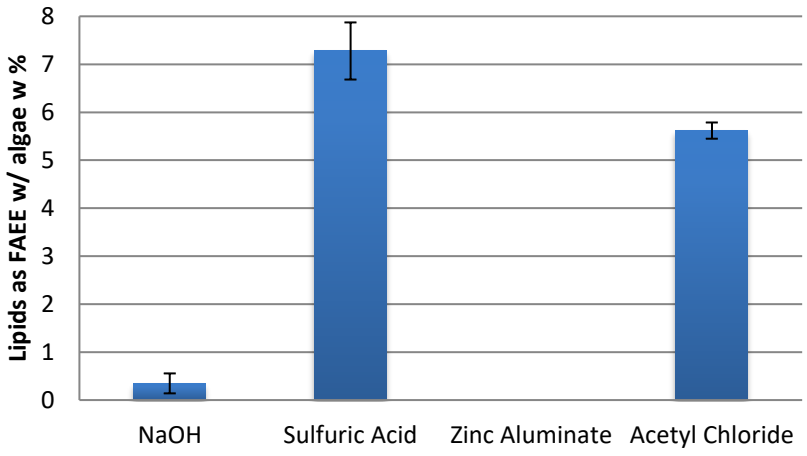

Figure 4.19. Direct transesterification of dry algae with different catalysts

Sodium hydroxide (NaOH) is a alkaline catalyst, largely used in the literature (Atadashi et al., 2013), but when used directly for transesterification only 0.4 ± 0.21% wt. was achieved. This demonstrates that the alkaline catalyst

was not able to penetrate the thick cell walls of the *C. vulgaris* and it is only likely to be efficient with an associated disruption method or in the biomass oil (Teo et al., 2014, Chen et al., 2012). Other possible reason for this result is the presence of FFA that reacts with the alkaline catalyst to form soap (as described in section 2.7.1).

Zinc aluminate ($ZnAl_2O_4$), provided by the Federal University of Bahia, was unable to produce biodiesel direct from the biomass and no yield was detected. It has been used before to produce biodiesel with the utilisation of CO_2 as co-solvent (Alves et al., 2013) and it is concluded that these positive results are due to the acidic environment produced by the co-solvent that acted as a Lewis acid catalyst and helped in the transesterification (Saharay and Balasubramanian, 2006).

Acid catalysts were shown to be the most efficient for *C. vulagris*. Sulphuric acid had the best performance with a production of 7.3 ± 0.60% wt. lipids as FAEE while the acetyl chloride obtained 5.6 ± 0.17% wt. Acid catalyst have demonstrated potential for the complete transesterification of the lipids from *C. vulgaris*. This better performance of the acid environment can be due to the wall cell of the algae and the amount of FFA present in this feedstock, while the alkaline catalyst would work better for the transesterification of the triglycerides (Laurens et al., 2012). The converted products by acid catalyst was assumed to be the total possible FAEE yield from *C. vulgaris* cultivated during this research.

With these results, the lipid content of the algae was assumed to be 7.3% wt. as FAEE of its weight. This value is low compared to other strains cultivated to produce biodiesel, which can achieve a value of 40% wt. (Stephenson et al., 2010), but it is understandable considering that the algae was cultivated for other purposes and this value can be expected to increase if cultivated with nitrogen stress (Singh et al., 2011).

The FAEE presented in the algae used in this study is mainly ethyl linolenate (62% wt.) follow by ethyl linoleate and palmitate. The FAEE profile is presented in Table 4.5.

Table 4.5. FAEE profile of *C. vulgaris*

FAEE		Mass (%)
C16:0	Ethyl Palmitate	16.71
C18:0	Ethyl Stearate	0.30
C18:1	Ethyl Oleate	3.00
C18:2	Ethyl Linoleate	18.05
C18:3	Ethyl Linolenate	61.95

4.3.4.3. Carbohydrates

With the amount of moisture (15% wt.) and ash (mineral) (10.22% wt.) determined by TGA, protein (36.24% wt.) and lipids (7.3% wt.) measured; it was possible to estimate the carbohydrates quantity using Equation 4.3. The total carbohydrate in the biomass was 31.2% wt.

The high carbohydrate content in the algae is mainly due to cellulose in the cell walls and can be converted to fermentable sugars (Chen et al., 2013). This

profile identifies the potential even more of the use of algae for biorefineries in the integrated production of different fuels and products.

4.4. Conclusions

The complete biomass characterisation was concluded and *Chlorella vulgaris* cultivated in BBM Media was selected to continue the following works in supercritical ethanol transesterification.

It was seen that with different growth methods, different characteristics for the same algae strain are obtained. The algae cultivated in the f/2 Media presented a contamination with another strain (*Pennales diatoms*) with high silica concentration (revealed in SEM and GC-MS analyses) and was discarded for future research.

Elemental and chemical analyses were conducted on the algae cultivated in BBM Media after drying process. It contained 47% carbon, 7% hydrogen, 8% nitrogen, 30% oxygen, 0.1% phosphorus and 7% of other elements in mass basis. These gave a calculated HHV of 21.95 $MJ \cdot kg^{-1}$ which demonstrates the high potential this technique has for fuel production.

The TGA analysis showed a high volatile matter (60% wt.) in the algae and demonstrated that these components started to decompose around 200°C which is a lower value than would be experienced in the reactor, hence some decomposition of the algae during the direct transesterification in supercritical ethanol would be expected.

The proteins, lipids and carbohydrates were also measured. This quantification is important to check the feasibility for the products in biorefineries. Proteins can be sold direct to the market while lipids and carbohydrates can be used to generate fuels such as biodiesel and ethanol, respectively.

Cell disruption methods were tested in order to improve the efficiency of the extractions. Ultrasonication revealed to be the best method to disrupt the cells of *C. vulgaris*; yields of 36% wt. proteins and 6% wt. lipids were obtained following this procedure.

Protein content was quantified by Lowry Method from dried algae incubated for 20 minutes in LS with a concentration of 3.4 $mg \cdot mL^{-1}$ and treated in Us for 60 minutes (submitted to 210 MJ) which gives a nitrogen factor of 4.02 for direct estimation from the elemental analysis.

The maximum lipids extraction was achieved using a solvent mixture of methanol:chloroform in ratio 1:2 v/v. When tested for direct transesterification to FAEE, the maximum conversion was 7.3% wt. using an acid catalyst (sulphuric acid). The FAEE profile exposed a high amount of linolenic acid (more than 60% wt.) followed by linoleic acid (18% wt.) and palmitic acid (16% wt.) which shows a potential for production of a high quality biodiesel. The lipid content was really low when compared to the literature, but this strain was not cultivated with the purpose to maximise this component. In spite of this, these algae were further used for this study and for biodiesel production.

5. Direct Biodiesel Production in Supercritical Ethanol

5.1. Introduction

The new route proposed in this work is the direct transesterification and esterification of non-dried algae in supercritical ethanol (SC-EtOH).

Chlorella vulgaris cultivated in Bold Basil media, was characterized with an amount of 7.3% wt. as FAEE in the previous chapter, was used for the direct biodiesel production.

Ethanol was selected because it is environmentally better than methanol, since it can be produced from renewable sources. The use of supercritical alcohol allows the miscibility of oil with the solvent, which is limited under non-supercritical conditions, leads to an increase in the reaction rate (Tan and Lee, 2011). The supercritical point of ethanol is at 240.9°C and 61.4 bar where its density is 276 $g \cdot L^{-1}$ (Jessop and Leitner, 2010).

In the literature, the direct transesterification of algae in SC-EtOH has been carried out in a batch reactor by Reddy et al. (2014) that used dry *Nannochloropsis salina* with 52% wt. lipids as FAME. The use of SC-EtOH for direct transesterification of algae was also studied by Jin et al. (2014) who reported the effect of Lewis acid (mainly $ZnCl_2$) on *Chlorella pyrenoidosa*. Both studies used batch reactors with the algae initially dry and not direct from harvesting. This study aimed to use algae with high water content direct from harvesting for FAEE

production in a flow reactor that could lead to improvements in conversion due the intensification of heat transfer because of its design.

The tests were initially carried out in the batch reactor to explore the reaction behaviour and then they were performed in the flow reactor, the main aim of this research.

5.2. Materials and methods

For the supercritical direct transesterification (SCDT), a batch reactor and a flow reactor specifically designed for this study were used. The algae used were the *Chlorella vulgaris* cultivated in Bold Basil Media as described in Chapter 4 and adjusted to the desired concentration by centrifugation (J2 Beckman floor standing refrigerated centrifuge at 5,000 rpm for 30 minutes), removing or adding the necessary amount of water.

SCDT was carried out by adding the biomass with water and the solvent (ethanol) to the reactor, closing the lid and increasing its temperature to reach the desire conditions. In order to measure the FAEE yield it was necessary to separate the reaction products, so after the reaction, water and hexane (20 mL each) were added to the product and left to separate for 30 minutes (Figure 5.1). This volume was chosen as it was the minimum necessary to complete the separation due the high ethanol volume. The solution divided in two layers: a top organic non-polar layer (with the hexane and FAEE) and a lower aqueous layer (formed by the water, ethanol, glycerol and char).

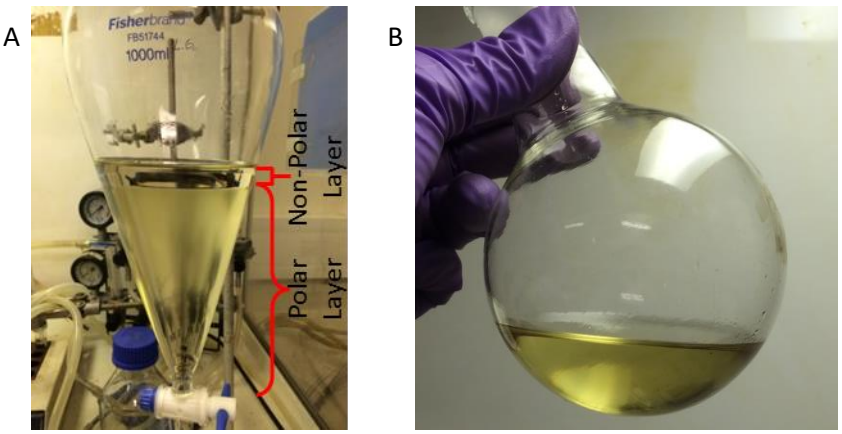

Figure 5.1. A) Separation between polar and non-polar layers; B) Hexane layer ready for concentration.

The hexane layer with the reaction products was further concentrated using vacuum evaporation and the product was analysed twice by gas chromatography (GC) with addition of methyl heptadecanoate as internal standard (IS). The water layer was also analysed by GC, but no FAEE compounds were detected. The gas chromatography method and calibration curves were described in the previous chapter.

A summary of the methodology is presented in Figure 5.2.

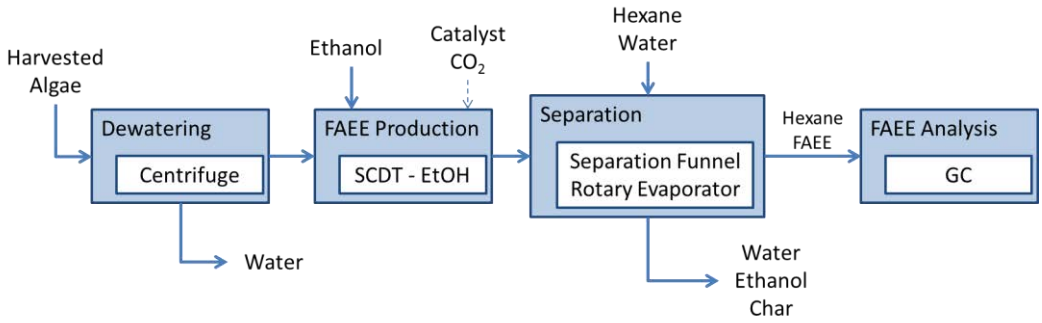

Figure 5.2. Methodology adopted for the experiments

After the reaction, the char was also studied using the CHN composition and by SEM images. The methodologies of these tests were the same as those used to characterise the biomass (sections 4.2.4 and 4.2.6).

5.2.1. Batch reactor

Batch SCDT was done in a 300 mL Parr bench top batch reactor 5500 series made from 316 stainless steel equipped with a variable speed magnetic drive stirrer and external electrical heater. Temperature and pressure in the vessel were monitored using a type K thermocouple (accuracy of ± 2°C) and a pressure gauge model 593HCPG (accuracy of ± 2.5 bar). The vessel was initially purged with nitrogen to create an inert environment. A schematic of the reactor is presented in Figure 5.3, where P and T represent pressure and temperature measurements.

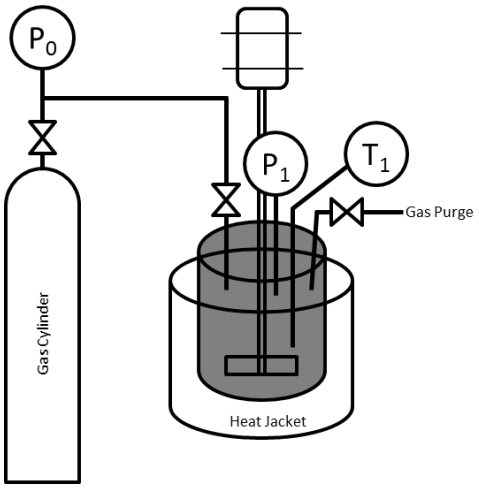

Figure 5.3. Batch reactor design

Diluted algae (100 mg in dry basis) were added to the reactor with 5, 10, 50 and 100 mL of ethanol and either 2.5, 5 or 7.5 mL of water to form a suspension according to the experiments described hereafter. The vessel was purged with nitrogen, the heater was turned on to the target set point temperature and the mixer (300 rpm) was started. The reaction time is counted from the time the reactor achieved the desired temperature (pressure is achieved with the

increasing of temperature). After the required reaction time was reached, the reactor was quenched in an ice bath to end the reaction.

The ethanol amount necessary for maximum FAEE yield was studied in the batch process. An ethanol volume of 5, 10, 50 and 100 mL was added to each 100 mg of algae and 5 mL of water to give ratios of 1:50, 1:100, 1:500 and 1:1000 w/v algae / ethanol. The influences of water, retention time, the use of catalyst and co-solvent (CO_2) on the reaction were also studied. The relationship between the temperature and water content variables was determined through a central composite design (CCD) with the conditions presented in Table 5.1.

Table 5.1. CCD Variables in the batch reactor with 100 mg of algae in 50 mL of ethanol and 15 minutes retention time

Variables	**Units**	***Level***		
		-1	***0***	***+1***
Temperature	*℃*	250	275	300
Water Content	$mL \cdot g^{-1}$	0.25	0.5	0.75

The necessary time for the maximum biodiesel yield was also studied. The retention time was measured after the reactor achieves 270°C with an initial pressure of 10 bar due to the addition of nitrogen (by the time the reaction achieved the desire temperature the pressure was between 90 and 100 bar in all experiments). 100 mg of algae in 5 mL H_2O and 50 mL of ethanol were used for these experiments. The time required for the maximum biodiesel yield was studied, separately from the temperature and water content, by varying the reaction time up to 12 hours.

Triglyceride palmitate and palmitic Acid were analysed in order to understand the behaviour of the pure compounds in the supercritical ethanol transesterification and esterification in the presence of water. They were purchased from Sigma Aldrich in their pure form (≥99%) and were tested in the batch reactor in line with the ratio previous found in the algae (between 1:500 and 1:1000 w/v TG or FFA / ethanol).

The catalyst tested was the zinc aluminate ($ZnAl_2O_4$) provided by Federal University of Bahia – Brazil, and described in Chapter 4. The catalyst was added in 2 proportions, 5 and 10% w/v of solvent, following the literature (Alves et al., 2013). Its influence was also tested at subcritical temperature (230°C).

The addition of a co-solvent was also tested by adding CO_2 to the batch reactor instead of using nitrogen to provide an initial pressure of 10 bar.

After understanding these SCDT parameters, a flow reactor was designed in order to improve yield and investigate scalability.

5.2.2. Flow reactor

The flow reactor was designed by following the literature (Vieitez et al., 2008, Velez et al., 2012) with some adaptation for the biomass feedstock. The final version is presented in Figure 5.4.

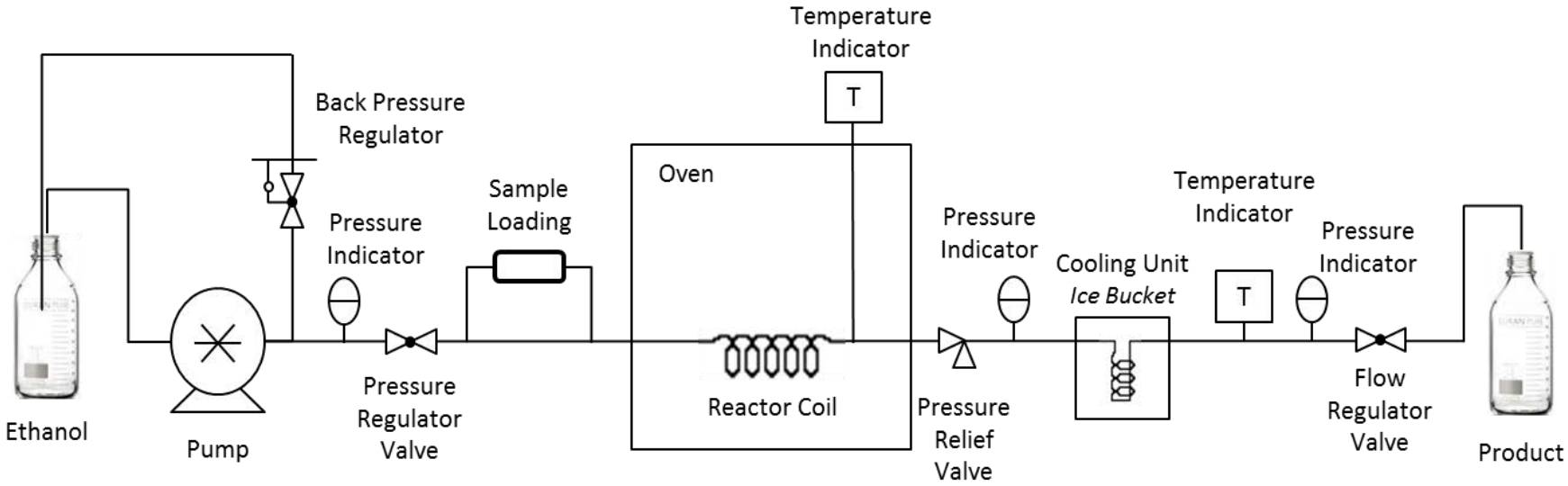

Figure 5.4. Flow reactor design

The system was built with stainless steel Swagelok tubing with ¼'' outside diameter (ID = 0.46 cm). The pump used was a HPLC Pump (Scientific Systems - Series III; Isocratic, Cat n. 06-656-261) and the oven was a GC oven (Hewlett Packard HP, 5890 series II). The 15 mL "sample loading" cylinder was built with ¾'' tubing, the coil reactor had a volume of 120 mL (5 m long) and the cooling system 30 mL (1.5 m longer ¼'' stainless steel Swagelok tubing in an ice bucket). Retention time varied from 10 to 40 minutes. Calculations for the cooling system are presented in Appendix 4. Thermocouples type K and pressure gauges type S were used for temperature and pressure measurement (accuracy ±0.80°C and ±1.5% bar). A flow metering valve (Hoke, 1656G4YA) was installed in the end of the system to control the flow and also controlled the pressure in the reactor. The system also had a back pressure regulator (Swagelok, KPB Series) installed just after the pump to ensure that the pressure did not increase more than the desired value and a relief valve (Swagelok, SS-4R3A, spring designator D adjusted to 140 bar) was connected after the reactor for safety reasons.

The sample was inserted after the pump because of the particle size of the diluted algae, as the pump was only able to convey the solvent with no particles. Another adaptation was made to the back pressure regulator that was obstructed by the char if installed in the end of the system. This problem was already verified by Deadman et al. (2015), they suggest a new model of back pressure regulator and presented that the main methods adopted are the manipulation of the chemical conditions, such as the use of multiphasic systems, the change in

concentration of reagents or using porous material to immobilise the components, and the use of mechanical agitation or ultrasound for forcing the dilution of the solids before the regulator. A strainer or filter also can be installed to protect the pressure regulator (Mankenberg, 2017). In the used design in this research, the back pressure regulator worked more as a safety valve for the pump than an actual pressure control. The pressure is being controlled by the pressure regulator (Swagelok, KHP Series) installed after the pump, as suggested by (Bolger, 2017), and also by the flow valve in the end of the process.

The sample containing the diluted algae was inserted in the “sample loading” cylinder and then ethanol was pumped into the system where it reached supercritical conditions inside the reactor coil. The final products were collected at room temperature and pressure after passing through cooling unit and flow regulator. The entire system is presented in Figure 5.5.

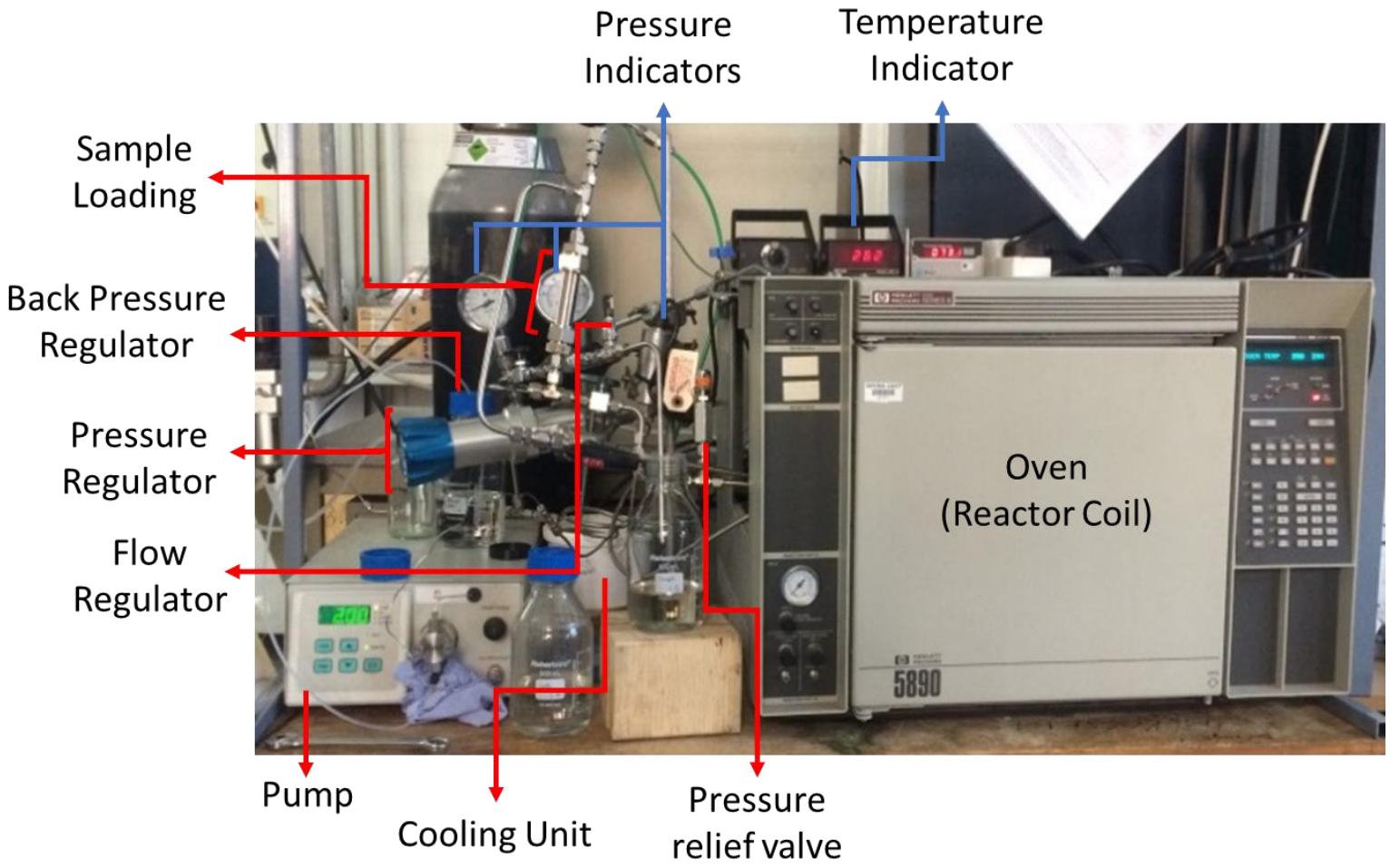

Figure 5.5. Image of the entire system

Four parameters were studied: temperature, pressure, water content and retention time (by the flow rate). The levels of variation in the CCD are presented in the table below.

Table 5.2. CCD variables in flow reactor

Variables	Units	Level		
		-1	***0***	***+1***
Temperature	*°C*	250	260	270
Pressure	*Bar*	65	75	85
Water Content	*mg Algae·mL^{-1}*	4	6	8
Flow	*mL·min^{-1}*	3.55	5.1	6.65

Temperature levels were defined starting in the subcritical temperature (250°C) and going until the temperature tested in the batch reactor (section 5.3.4) (270°C). Pressure was also based on the supercritical point of the ethanol, however this fluctuated as the flow control was imprecise and varied by ± 5 bars because of problems in the design. The water content was limited by the minimum water necessary to dilute the algae and was increased by 2 mg·mL^{-1}; above this value the flow rate was limited by the pump which had a maximum flow of 10 mL·min^{-1}, so the values were divided to have 5 levels.

5.2.3. Design of experiments (DoE)

Experiments performed in the batch reactor and in the flow reactor were designed using a central composite design. A central composite design is the most commonly used surface response design. It is a fractional factorial design with centre points (0 in all levels), cube points (±1 in all levels), hybrid points (±1 in one

level) and axial points (±α in one level) that allow the estimation of curvature. A visual representation with three input parameters is presented in Figure 5.6.

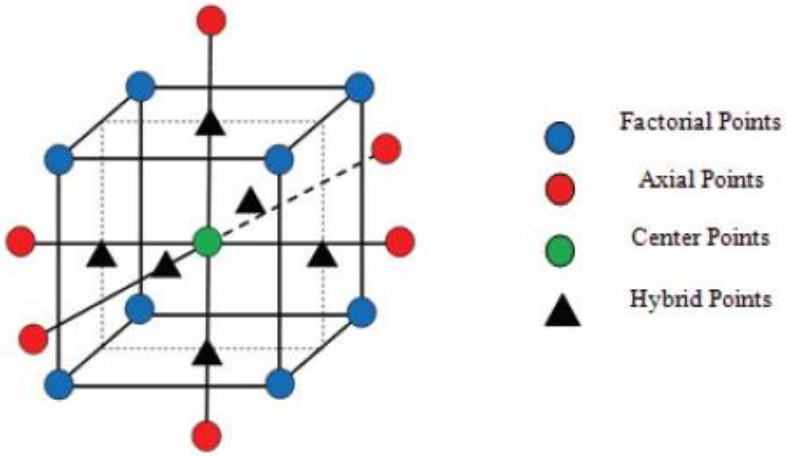

Figure 5.6. CCD design (Pontes et al., 2010)

In this design, parameters levels (-1, 0 and 1) are defined in order to build a quadratic model without using a full factorial set of experiments. Four factors with three levels would demand 81 experiments without considering the repetitions in the full factorial analysis while in the CCD only 30 experiments would be necessary due statistic interactions (Minitab, 2016). In both CCD the experiments were done in a random order and the generated model was analysed by the probability of obtaining the results (p-value) and the coefficient of determination (R^2) that indicates how much the results fit to the model.

5.3. Results and discussion

The initial experiments were done in a batch reactor to test the most important parameters (temperature, ethanol oil ratio, water content and retention time). Pure triglyceride and fatty acid were used as reference

compounds to check the influence of other parameters associated with the algae (such as influence of the cell wall and quantities of FFA and TG). These data were then used to design the flow reactor to produce biodiesel.

5.3.1. Products from direct transesterification

As described in the chapter before (section 4.3.4.2), base line transesterification (using sulphuric acid as catalyst) gave a maximum yield of 7.3 ± 0.60% wt. g FAEE on a dry mass basis, and this value was assumed as the maximum that the SC-EtOH transesterification could achieve.

Products were analysed using GC and it was possible to identify the same five main ethyl esters as seen in the batch transesterification (ethyl palmitate, ethyl oleate, ethyl linoleate, ethyl α-linolenate, ethyl stearate) in different ratios, as presented in Table 5.3.

Table 5.3. FAEE profile of *C. vulgaris* obtained from catalyst transesterification

FAEE			Mass (%)
C16:0	$C_{18}H_{36}O_2$	Ethyl Palmitate	65.4 ± 8.92%
C18:0	$C_{20}H_{40}O_2$	Ethyl Stearate	3.1 ± 2.77%
C18:1	$C_{20}H_{38}O_2$	Ethyl Oleate	4.1 ± 2.20%
C18:2	$C_{20}H_{36}O_2$	Ethyl Linoleate	8.7 ± 3.02%
C18:3	$C_{20}H_{34}O_2$	Ethyl Linolenate	18.7 ± 5.06%

In contrast to the catalyst transesterification (CT) (Table 4.5), the SCDT product had ethyl palmitate as the major component (> 65% wt.) followed by linolenate and linoleate (Table 5.3). Possible reasons for this behaviour are the degradation at the process temperature or non-conversion of the linolenic acid.

One surprise revealed by the GC-MS is the amount of phytol and isomers that were not seen from the CT. A chromatogram example is presented in Figure 5.7 and a complete GC-MS of the sample is presented in the Appendix 5.

In the chromatogram, such as the one presented below, it is possible to see the five main ethyl esters, the internal standard (IS) and the phytol peaks. The smaller peaks were not identified by GC-MS or when compared to a commercial mix of FAEE. The literature suggests that these peaks are related to degradation products from proteins, carbohydrates and hydrocarbons (Kasim et al., 2009, Levine et al., 2010).

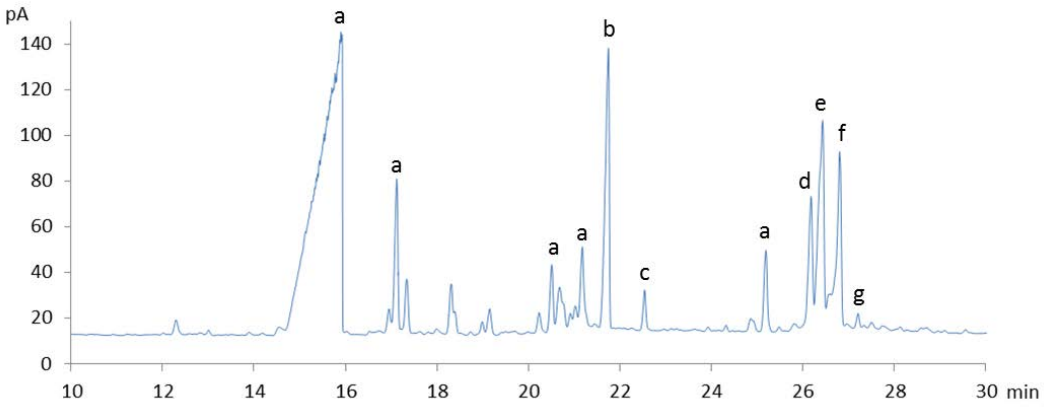

Figure 5.7. SCDT Gas chromatogram example (a - Phytol; b - Ethyl palmitate; c - IS; d - Ethyl linoleate; e - Ethyl α-linolenate; f - Ethyl oleate; g - Ethyl stearate)

Phytol ($C_{20}H_{40}O$ – 3,7,11,15-tetramethyl-2-hexadecen-1-ol) is released in the hydrolysis and degradation of chlorophyll and is not produced by the CT as there is no water present in the reaction. It can be a useful product with a number of industrial applications such as an additive in the medicinal and food sectors (de Moraes et al., 2014). Phytol, as other heavy oils, can also be used as a blend stock

in diesel to improve its performance (Rajesh Kumar and Saravanan, 2016). Ramírez et al. (2014) reported similar engine efficiencies and emission characteristics as diesel with a blend of up to 20% v/v phytol.

5.3.2. Effect of ethanol quantity in the reaction

The effect of ethanol volume was investigated in the batch reactor. During the process of transesterification in supercritical ethanol, the amount of reagent necessary is expressed by the relationship between algae:ethanol (w/v) (Reddy et al., 2014) or oil:ethanol (w/v or molar) (Gui et al., 2009).

It was suggested previously that the ratio oil:ethanol should be around 1:40 (Vieitez et al., 2008), but due to the algae structure and the amount of water (for undried systems) the volume of ethanol would need to be greater. Ethanol is used to dissolve the sample, decrease the supercritical point of the mixture and shift the reaction to FAEE production. Dry algae weight per ethanol volume was also used as basis for the ratio calculations and the maximum biodiesel yield was found for a ratio of 1:4.8 (Jin et al., 2014) to 1:9 w/v on a dry basis (Reddy et al., 2014, Patil et al., 2011). Conversion of algae (100 mg) was also tested using acid transesterification (1.8% v/v H_2SO_4) with methanol (4 mL) and gave the maximum yield of 84% (Wahlen et al., 2011).

All experiments were undertaken with the same amount of algae (100 mg) and water (5 mL) at 270°C for 15 min with a pressure over 90 bar. The ethanol content varied from 5 mL (1:1 v/v to water) to 100 mL (when the yield started to decrease). Figure 5.8 shows the results obtained.

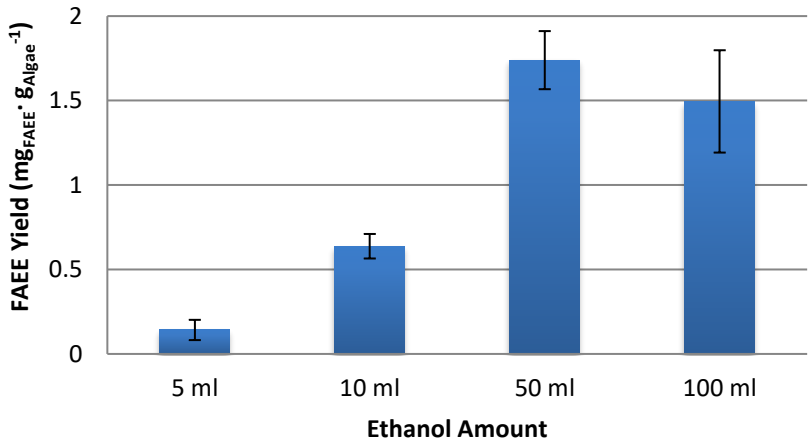

Figure 5.8. Influence of ethanol quantity on biodiesel yield in the presence of 5 mL water and 100 mg algae for 15 minutes

It was observed that the use of 50 mL ethanol per 100 mg algae gave 1.74 ± 0.17 mg FAEE·g^{-1} algae a greater result when compared to the other tested ethanol amount, so 50 mL of ethanol was used in the further experiments. 100 ml gave similar results, but as it is higher amount and then higher cost, it was decided to use the 50 ml.

It was also observed that decreasing this amount, the ethanol was insufficient to act as solvent, catalyst and reactant. This identifies the influence of the water and the amount of ethanol necessary and has not been reported before. Vieitez et al. (2008) tested the influence of high volumes of water (until 10% v/w in relation to ethanol) with soybean oil. In their study, the water increased the process efficiency at a low flowrate (0.8 and 1.0 mL·min^{-1}) and its presence was negative at higher flowrates, while keeping the molar ratio of oil and ethanol (1:40 molar ratio) constant. The molar ratio between oil and ethanol would also change if the algae had a larger oil content, so tests with algae

cultivated for biodiesel production (that could achieve 40% wt. lipids as FAEE) need to be studied in the future.

By not drying the algae generates a high consumption of ethanol. The larger volumes of ethanol are necessary to decrease the supercritical point of the mixture and guarantee the action of the ethanol as extraction solvent and FAEE production reactant. Wahlen et al. (2011) needed to increase the amount of solvent (methanol) from 2 to 4 mL for their batch process in order to maintain the same FAME production from wet algae (with 100% w/w) when compared to dry algae but could not achieve the same amount produced with 400% wt. water. It was therefore questioned if it is worth the extra solvent cost instead of drying the algae. This is analysed briefly in Chapter 6.

It is important to assess further ways to reuse the ethanol added or decrease this ethanol amount. It was suggested previously that by adding hexane in the reaction the ethanol could be decreased (Pérez et al., 2013) but this could require additional washing steps. Another solution would be to consider producing the FAEE in two steps firstly by hydrolysis (reaction only with water to produce FFA from TG) and then by the SC-EtOH (Levine et al., 2010, Minami and Saka, 2006). Future studies are necessary in this area.

Knowing the proportions of water and ethanol and using the Peng-Robinson Equation of State with Wong-Sandler mixing rules, the supercritical point of the mixture was calculated (Ramirez, 2016). The figure below shows this point as 255°C (528 K) and 74 bar. It is important to highlight that the supercritical

point of the mixture is higher than pure ethanol which would imply an increase energy demand and costs of operation, but lower than the Tc and Pc of water.

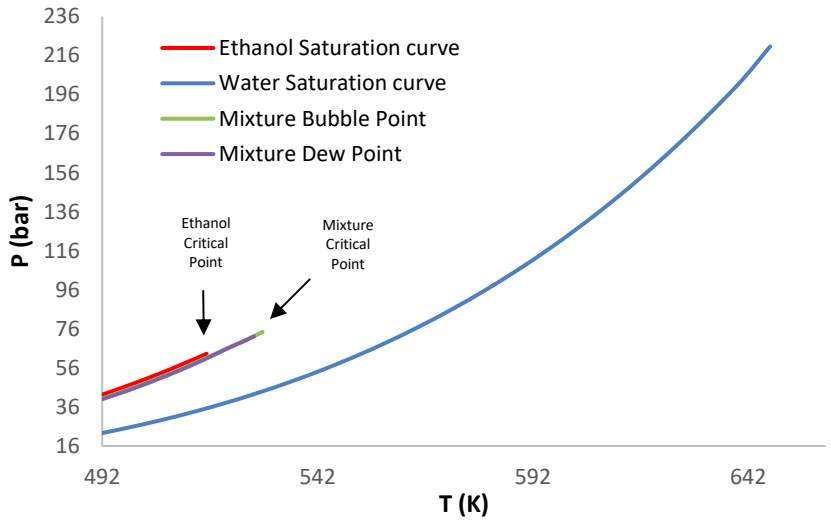

Figure 5.9. Mixture critical point representation

All the experiments in the next sections considered these findings and did not consider the oil or biomass present in the reactor. These are present in low percentages when compared to the volumes of ethanol and water and simplified the calculations.

5.3.3. Temperature and water content

The temperature and water content were studied as described in (section 5.2.1). A regression model was created from which further yields could be predicted. The summary of experiments is presented in Table 5.4.

Table 5.4. DoE batch reactor

	Parameters		Results	
	Temp (°C)	Water Content (mL·g^{-1}Algae)	FAEE (mg·g^{-1}Algae)	Conversion (%)
13	250	0.25	1.16	1.59
6	250	0.5	1.64	2.25
3	250	0.75	1.12	1.53
7	275	0.25	1.28	1.76
8	275	0.5	1.76	2.41
9	275	0.5	1.83	2.5
10	275	0.5	1.8	2.46
11	275	0.5	1.73	2.37
12	275	0.5	1.76	2.41
4	275	0.75	1.11	1.52
2	300	0.25	1.92	2.63
1	300	0.5	2.17	2.97
5	300	0.75	1.5	2.05

The experiments generated the quadratic regression model shown in the equation below and yielded the results presented in Table 5.5.

$$FAEE\ Yield\ (mg\ FAEE/g\ Algae) = 16.42 - 0.1391\ T + 1.220\ W + 0.000287\ T^2 - 0.08500\ W^2 - 0.001500\ T\ W$$ **Equation 5.1**

Where, T is the reaction temperature (°C) and W is water (mL) per 0.1 g of dry algae. The statistical fit is shown in Table 5.5.

Table 5.5. CCD water content and temperature

Parameter	F-Value	p-Value
Temperature (°C)	222.62	0.000
Water content (mL·0.1 g^{-1})	32.89	0.001
Temperature^2 (°C^2)	42.73	0.000
Water content^2 (mL^2 0.01 g^{-2})	375.44	0.000
Temperature x Water content (°C mL·0.1 g^{-1})	16.94	0.004

Model Summary			
S	R-sq	R-sq (adj)	R-sq (pred)
0.0455657	98.93%	98.17%	94.59%

A response surface plot (Figure 5.10) was constructed to represent the interaction between yield, temperature and water amount. It is possible to see that the FAEE yield increases with reaction temperature and achieves the maximum at 300°C.

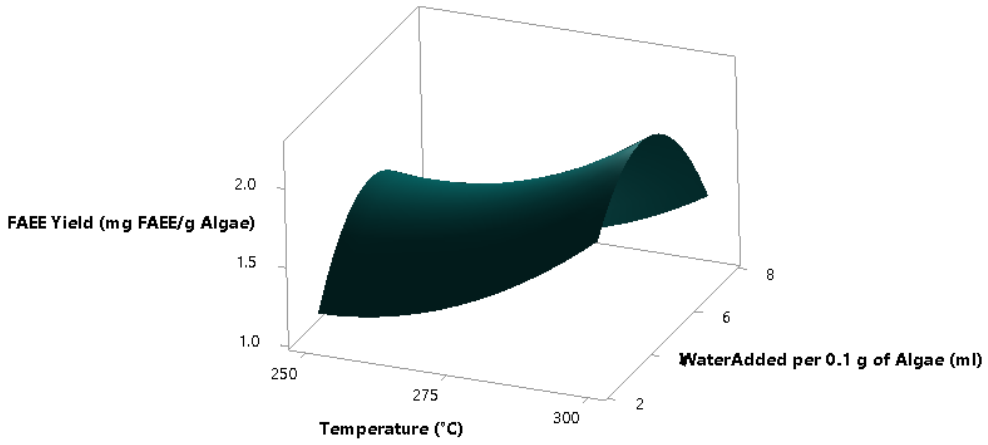

Figure 5.10. Effect of temperature and water on FAEE yield response surface plot

The water content however gave an optimum at 4.52 mL of water added per 0.1 g of algae. The water can be responsible for the transesterification and hydrolysis of triglycerides and esterification of the FFA (Silva and Oliveira, 2014). Hydrolysis would help in the formation of FFA, and consequently, the production of FAEE would be faster by the esterification route. The simplified mechanisms are schematically shown in the figure below based on Kusdiana and Saka (2004). The FFA would act as an auto catalyst for the hydrolysis and esterification in the water (Minami and Saka, 2006). High water content would limit FAEE yield by hydrolysing the FAEE (Levine et al., 2010), this has been suggested by Jin et al. (2014) who showed that the biodiesel yield decreased from algae in the presence

of a catalyst when the moisture content was greater than 5% vol. suggesting the necessity of a dewatering process.

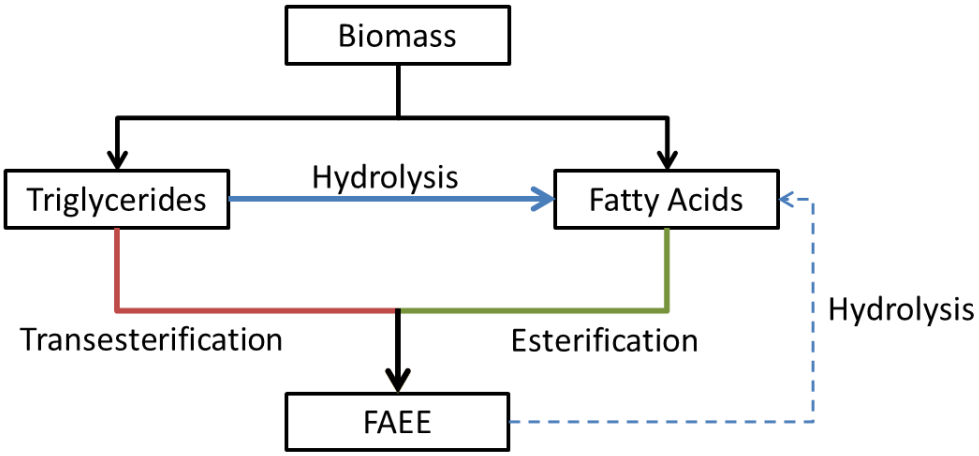

Figure 5.11. Mechanisms reactions in presence of water in supercritical ethanol reactor

This water effect is fundamental to the feasibility of algae as a feedstock, since it is known that in alkaline transesterification (the current commercial route for other feedstocks) the reaction is impaired by water, so an energy intensive drying process is necessary. In supercritical EtOH the drying process could be eliminated as long as the water content is < 45 mL of water per g of algae (achieved after a dewatering step).

The effect of temperature agrees with previous research, where higher temperatures produce higher FAEE yield (Vieitez et al., 2008, Trentin et al., 2011). The increase in FAEE yield is probably due to the increased solvating power seen at high temperatures. 300°C was the maximum temperature tested due the limitation of the reactor used and the literature suggests that algae and FAEE degrade after this point (Reddy et al., 2014). The degradation increases in higher

temperatures with longer retention times (Olivares-Carrillo and Quesada-Medina, 2011).

5.3.4. Temperature and reaction time

The influence of the temperature was tested in conjunction with a longer reaction time, as it has been reported that the efficiency could increase in lower temperatures (Silva et al., 2014). The experiments were carried out with 100 mg of algae in 5 mL H_2O and 50 mL of ethanol. The reaction time is based on when the reactor achieved the target temperature. A temperature time profile is shown in Figure 5.12. Nitrogen was added to 10 bar pressure at the start of the heat cycle to give a final pressure of 100 bar, which is above the critical mixture pressure.

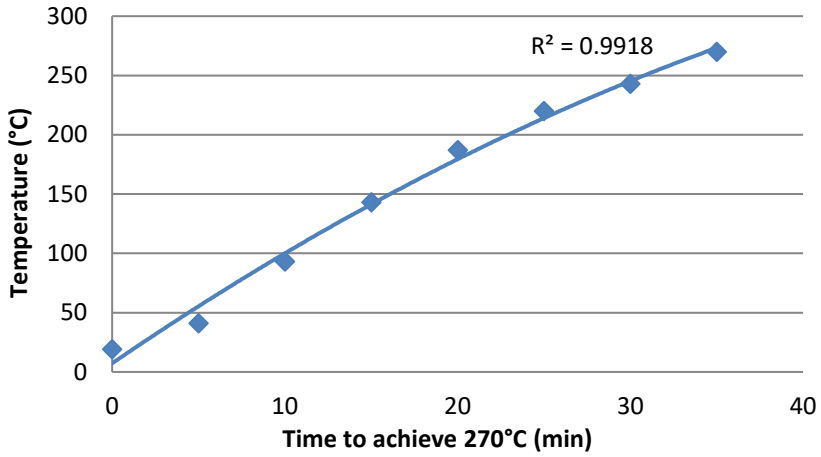

Figure 5.12. Reactor heat-up profile from the time the heaters are switched on until the time to achieve 270°C

It can be seen in Figure 5.13 that at 300°C there was a higher conversion compared to 270°C after 15 min of reaction (as suggested in the section before), however after four hours the lower temperature gave better results than the higher one. One possible explanation is that FAEE degraded over the longer time

period at 300°C as reported in the literature (Reddy et al., 2014). Thermal cracking of FAEE at 300°C for 15 min has been demonstrated by Olivares-Carrillo and Quesada-Medina (2011).

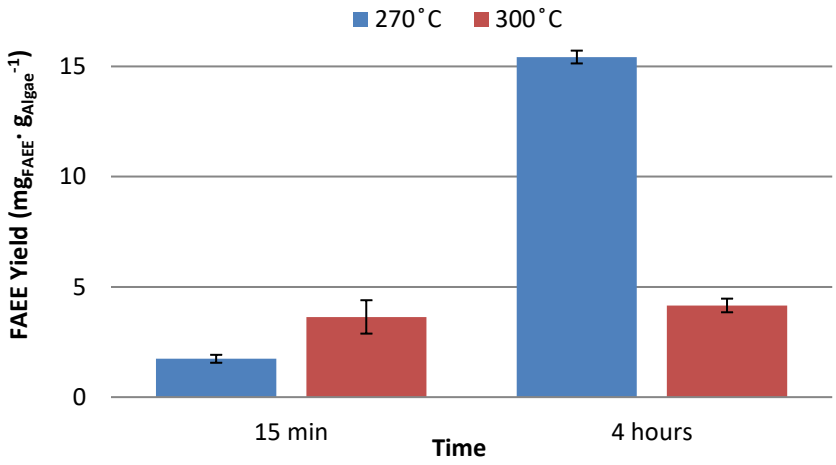

Figure 5.13. Relation between temperature and retention time in the reactor

5.3.5. Retention time

After noticing the influence of the retention time it was necessary to check when the reaction would achieve its maximum. The transesterification reaction was carried out at the same conditions presented earlier at 270°C, 100 bar and 300 rpm with a retention time up to 12 hours. The negative times were obtained during the heating up process with the temperature lower than the required set point. The results are presented in Figure 5.14.

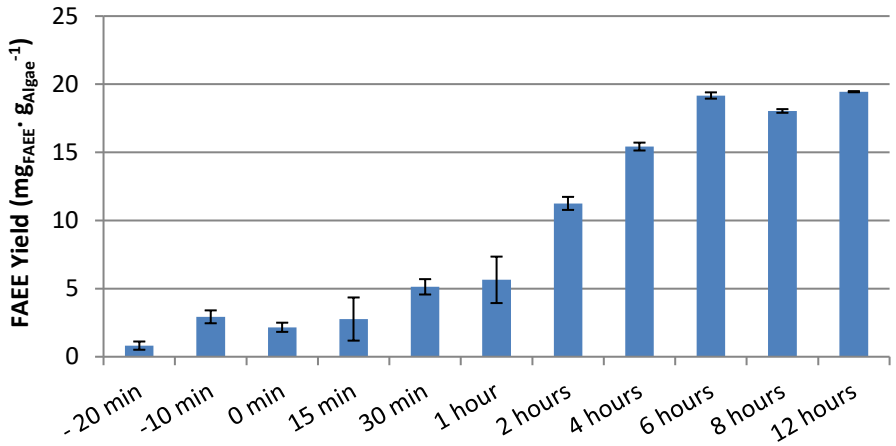

Figure 5.14. Effect of retention time on FAEE yield at 270°C

It is possible to see some conversion prior to the set-up point temperature being achieved. At -20 min time the temperature was 200°C and at -10 min was 240°C.

The batch experiments illustrated that the conversion does not achieve a maximum until 6 hours where the yield was almost 20 mg FAEE·g^{-1} algae. After this time, the amount of FAEE produced is relatively stable even up to 12 hours. This represents a conversion of 26% wt., much lower than expected when compared with CT, but is similar to some values reported in the literature for the conversion of pure oil at similar temperatures (Gui et al., 2009).

If pure oil is used, the temperature required for its conversion is > 320°C with some papers suggesting around 400°C (Silva and Oliveira, 2014, Tan et al., 2011). It is possible therefore to infer that the algae cell structure of the algae impedes the full transesterification. The cell structure impacts the retention time, and it was reported in the literature a maximum yield after 30 minutes with pure

oil (Ngamprasertsith and Sawangkeaw, 2011) or 10 minutes with *Nannochloropsis Salina* that has thinner wall cells (Reddy et al., 2014).

In order to ascertain the total potential of the SCDT reactor in these configurations, control measurements were carried with pure triglycerides (TG) and free fatty acids (FFA) and are reported in section 5.3.6.

5.3.6. Modelling with pure triglyceride (TG) and free fat acid (FFA)

Triglyceride palmitate and palmitic acid were used to test the reaction of biodiesel production whilst excluding the influence of the algae cell structure during the process.

The experiments were performed for a reaction time of one hour. It should be noted that after this time the algae gave a conversion of only 3.8% wt. yield. The results for the FFA yields are shown in Figure 5.15.

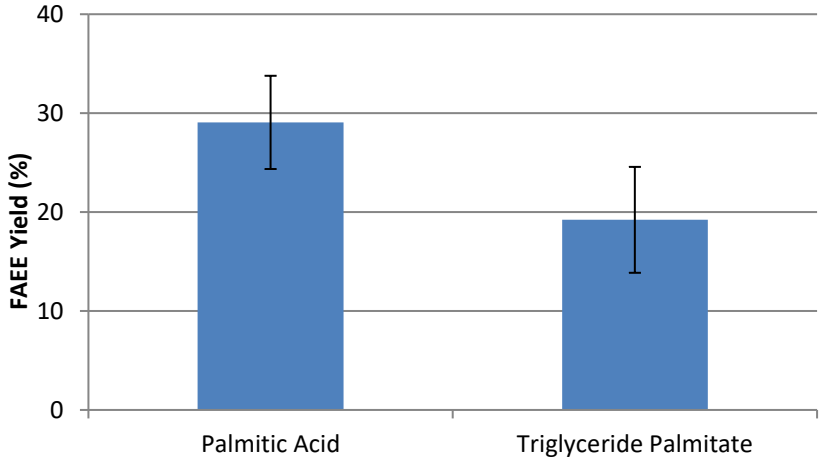

Figure 5.15. Reaction of FAEE production from palmitic acid and triglyceride palmitate at 60 min, T = 270°C, P = 100 bar, 300 rpm in SC-Ethanol

For the pure fatty acid or triglyceride tests, the conversion increased however the reaction was incomplete. It is possible therefore to understand some aspects of the reaction. The increased yield suggests that the algae structure has some influence on the reaction. It may be due to the solvation power of the supercritical EtOH extracting the oil and suggests the need for longer processing times and the use of co-solvent for the complete reaction.

The tests with the pure compounds also indicate that in the supercritical EtOH environment free fatty acids are converted to a greater extent than the triglycerides (29.1% compared to 19.2% wt.). This is due to the difference in the conversion mechanisms, since from FA to FAEE is a single step reaction, while TG to FAEE involves three steps. This is an important finding as usually a high FFA content decreases the biodiesel yield in catalytic transesterification as a result of the saponification reaction, whereas with the supercritical solvent process it would also form FAEE. This suggests that this technology could also be used for feedstocks with a higher FFA content such as waste oil or *Jatropha curcas* oil (Silva et al., 2014).

It can be observed that the use of supercritical ethanol with wet algae did not achieve similar yields when compared to the pure oil. It indicates that the cell wall and lipids extraction of the lipids from the algae are limiting factors to produce FAEE. This highlights further work is essential to identify methods to increase its efficiency, such as the use of co-solvents, pre-treatments or a two-step reaction (oil extraction and then transesterification) (Minami and Saka,

2006). Levine et al. (2010) using a batch reactor with algae oil obtained a yield of 79.2% wt. in a 120 minutes reaction at 275°C with a ratio of 1:2.3 w/w oil to ethanol and no water. Another solution to investigate would be the use of a flow reactor to improve the performance.

5.3.7. Addition of catalyst

In order to try to improve the yield produced in the transesterification reaction, zinc aluminate ($ZnAl_2O_4$) was added as catalyst. This catalyst was selected because of its performance in previous experiments in University of Bahia (Alves et al., 2013). Its performance was tested in two different ratios based on the work of Alves et al. (2013) and for two temperatures to check the performance also at subcritical temperature (230°C and 270°C). The retention time was 15 minutes, 300 rpm mixing and the composition the same reported in section 5.3.2 (100 mg algae to 5 mL of water and 50 of mL ethanol).

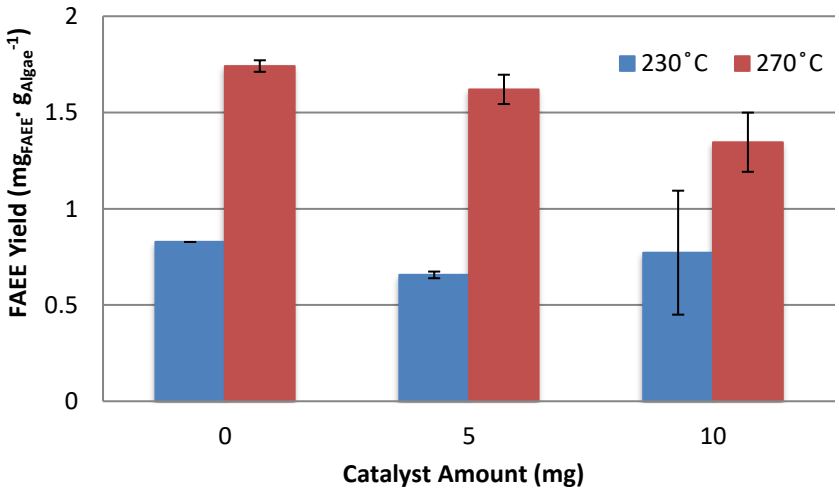

Figure 5.16. Catalyst ($ZnAl_2O_4$) influence on FAEE yield at 230°C and 270°C

The results show that the catalyst does not improve the reaction yield at 270°C, but leads to a decrease. It can be explained by the presence of water in the algae that inhibited the action of catalysts (Liu et al., 2006, Jin et al., 2014) and by the mass transfer limitations that are inserted by the addition of one more element in the reaction.

This indicates that the catalyst does not perform its role in extreme or milder conditions. Alves et al. (2013) demonstrated that $ZnAl_2O_4$ improved the transesterification of waste frying oil at 200°C, so the catalyst could be used only at lower temperatures. NaOH catalyst was also tested before by Tang et al. (2007) in subcritical conditions (250°C) and showed an improvement in the yield with oil from *Jatropha curcas,* but there was no water present. The literature suggests that the addition of the catalyst would be useful for decreasing the extreme conditions of the supercritical alcohol for the oil transesterification without the addition of water.

This work demonstrated that the catalyst does not perform the same role in the presence of water that would inhibit its action. Another possible solution to improve the yield could be the addition of a co-solvent to the reaction.

5.3.8. Using CO_2 as co-solvent

The addition of a co-solvent could increase the performance of the SCDT process by changing the polarity of the main solvent and increasing the solubility of the lipids (CO_2 is non-polar as are the lipids). CO_2 is nontoxic (considered a "green solvent") and has a high diffusivity (Saharay and Balasubramanian, 2006).

CO_2 was added to the reaction mixture (100 mg of algae on a dry basis diluted in 5 mL water and 50 mL ethanol) to give an initial pressure of 10 bar. Figure 5.17 shows the FAEE yields with either nitrogen or CO_2 at 270°C for 15 minutes.

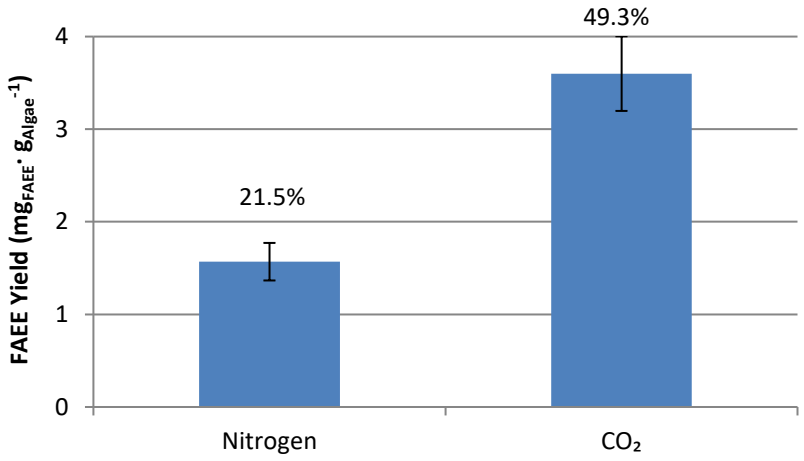

Figure 5.17. Effect of co-solvent addition on FAEE yields

The addition of CO_2 improved the reaction yield by 125% wt. (from 1.6 to 3.6 mg FAEE·g^{-1}algae) giving a conversion of 49.3%. This demonstrates the potential of CO_2 co-solvent in increasing the solubility between TG and solvent hence decreasing the mass transfer limitations (Silva et al., 2014). Trentin et al. (2011) reported similar results where the FAEE yield from soybean oil increased from 65% without CO_2 to 78% with CO_2 in a flow reactor at 300°C and 200 bar. The application of CO_2 would also decrease the mixture critical point (Kasim et al., 2009). CO_2 as co-solvent would also have an important role in the extraction of the oil from the algae (Dejoye et al., 2011). Supercritical CO_2 is a non-polar solvent as opposed to water and ethanol with a strong affinity for lipids (Sahena et al., 2009).

5.3.9. Flow reactor

The flow reactor was designed (Figure 5.4) and four parameters were selected for investigation (Table 5.2): temperature, pressure, water content and reaction time (from the flowrate). The pressure was included in the analysis since it is controlled by the pump, valve and regulator as opposed to the batch reactor where the pressure depends on temperature and the free volume available in the reactor for expansion on heating (De Boer and Bahri, 2011).

5.3.9.1. Design of experiment

The DoE was undertaken for a total of 30 experiments. Its order and results are presented in Table 5.6. The results are presented in mg of FAEE produced per g of algae and as a percentage calculated from the maximum yield produced by extraction followed by acid transesterification (7.3% wt. FAEE) calculated in section 4.3.4.2. The model was inconclusive and could not been trusted for identifying the best conditions for the reaction.

The highest yield (47.5% wt.) occurred at experiment 1 (260°C, for a pressure between 70 and 80 bar, an algae concentration in water of 6 $mg \cdot mL^{-1}$ and a flow of 2 $mL \cdot min^{-1}$). By the results, it is possible to make some observations. The temperature does not need to be the highest to improve the yield (higher conversions were obtained at 250°C and 260°C than 270°C and 280°C); the longest retention time improved the yield (with only one case where the opposite occurred (comparing experiments 24 and 28)); the pressure had an unexpected result where 65 bars showed to be better than higher pressures (some examples

are comparing experiments 12 with 24, 14 with 23, 5 with 17 and others); and the water content had no conclusive results, in some moments more water increased the conversion (23 and 28), decreased (12 and 13) or did not make difference (24 and 26).

Table 5.6. DoE flow reactor

	Parameters				Results	
	Temp (°C)	**Pressure (bar)**	**Algae Concentration ($mg \cdot mL^{-1}$ water)**	**Flow ($mL \cdot min^{-1}$)**	**FAEE ($mg \cdot g^{-1}$Algae)**	**Conversion (%)**
10	240	75	6	5.1	0.5	6.2
26	250	65	4	3.55	2.1	28.2
23	250	65	4	6.65	1.1	15
24	250	65	8	3.55	2.1	28.6
28	250	65	8	6.65	2.9	40
13	250	85	4	3.55	2	27.3
14	250	85	4	6.65	0.2	3
12	250	85	8	3.55	0.6	8.2
19	250	85	8	6.65	0.5	6.1
5	260	55	6	5.1	1.5	20.6
8	260	75	2	5.1	1.6	21.5
1	260	75	6	2	3.5	47.5
2	260	75	6	5.1	1.1	15.5
9	260	75	6	5.1	0.8	10.9
17	260	75	6	5.1	0.6	7.8
20	260	75	6	5.1	0.8	11
22	260	75	6	5.1	0.4	6.1
25	260	75	6	5.1	0.6	8.8
7	260	75	6	8.2	0.7	9.7
4	260	75	10	5.1	3	41
3	260	95	6	5.1	0.8	10.4
27	270	65	4	3.55	1.6	21.9
18	270	65	4	6.65	1	13.4
21	270	65	8	3.55	1.3	17.2
11	270	65	8	6.65	1.2	16.2
15	270	85	4	3.55	0.4	5.2
30	270	85	4	6.65	0.3	4.6
16	270	85	8	3.55	0.8	11.2
29	270	85	8	6.65	0.2	2.3
6	280	75	6	5.1	0.7	9.7

These results were analysed by a central composite design and the statistical results are presented underneath the regression equation model.

$$FAEE\ Yield\ (mg\ FAEE/g\ Algae) = \quad \textbf{Equation 5.2}$$

$$-36.3 + 0.437\,T - 0.266\,P - 0.18\,C - 1.98\,F - 0.00102\,T^2 + 0.00036\,P^2 + 0.0807\,C^2 + 0.1140\,F^2 + 0.00099\,T \cdot P - 0.00175\,T \cdot C + 0.00274\,T \cdot F - 0.00813\,P \cdot C - 0.00726\,P \cdot F + 0.0681\,C \cdot F$$

Where, T is the reaction temperature in the oven (°C), P is pressure (bar), C is concentration of algae per mL of water ($mg \cdot mL^{-1}$) and F is the flow rate controlled by the pump ($mL \cdot min^{-1}$).

Table 5.7. CCD flow reactor

Parameter	F-Value	p-Value
Temperature (˚C)	0.52	0.482
Pressure (bar)	0.39	0.544
Concentration ($mg \cdot mL^{-1}$)	0.01	0.933
Flow ($mL \cdot min^{-1}$)	0.56	0.468
Temperature^2 ($˚C^2$)	0.81	0.383
Pressure^2 (bar^2)	0.10	0.753
Concentration^2 ($mg^2 \cdot mL^{-2}$)	8.07	0.013
Flow^2 ($mL^2 \cdot min^2$)	5.82	0.030
Temperature*Pressure (˚C·bar)	0.44	0.581
Temperature*Concentration (˚C·$mg \cdot mL^{-1}$)	0.06	0.817
Temperature*Flow (˚C·$mL \cdot min^{-1}$)	0.08	0.779
Pressure*Concentration (bar·$mg \cdot mL^{-1}$)	1.19	0.293
Pressure*Flow (bar·$mL \cdot min^{-1}$)	0.57	0.462
Concentration*Flow ($mg \cdot min^{-1}$)	2.02	0.177

Model Summary

S	R-sq	R-sq (adj)	R-sq (pred)
0.594950	76.69%	51.71%	0.00%

The model had low significance (R-sq of 76.69%) and could not be applied to predict the correlation between the parameters and the best yield possible. The residual plots also indicate similar behaviour with the residual giving more than ± 0.5 (Figure 5.18).

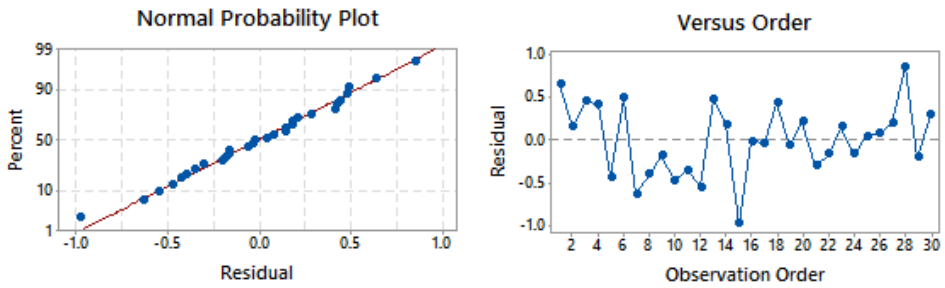

Figure 5.18. Residual plots for FAEE yield %

The DoE was repeated but the statistical significance was still low. The reasons for this could be the non-constant pressure in the flow reactor varied between 5 bar in the process.

Another factor could be due to the presence of algae particles in the feedstock. In the studied case, there were problems pumping the suspended algae solids and accumulated in the pump, valves or even retained on the tubing wall. A way to solve the particle problem in the design was to operate the system in a flow design where the solvent was pumped and mixed with the biomass after the pump. The back pressure regulator was installed just after the pump to control the pressure in the system with only a flow regulator at the outlet.

Within spite of the design problems the flow reactor showed potential to improve the direct transesterification reaction. Flow reactors were used

previously for direct transesterification of different plant oils, such as rapeseed and soy oil, while wet algae biomass has only been studied in batch (Reddy et al., 2014).

Temperature and reaction time has been largely studied by the literature as discussed before (section 5.3.4), but there little study on pressure as it is not typically controlled in a batch process. Pressure in a flow reactor was studied with rapeseed oil by Anikeev and Yakovleva (2012) that also identified the importance of this parameter. In their research, it was concluded that at higher pressure the yield would increase and it does not agree with the findings of this study. Further studies with a more stable system are required to understand the influence of each parameter.

Experiments were repeated at 260°C and 70 bar, at a concentration of 10 $mg \cdot mL^{-1}$ of algae in water with a solvent flow of 4.25 $mL \cdot min^{-1}$ giving a retention time of 20 minutes in the reactor. It was possible to obtain a yield of 36.0 ± 1.4% wt. FAEE. This value shows the improved potential of the flow reactor over batch, as it was not possible to obtain this yield in batch even after six hours.

This could be explained due to the intensified heat transfer in the process, since the temperature is constant in a coil reactor (not varied depending of the region as in the mixed bath reactor) and the surface contact area is larger for the same volume (Hartman et al., 2011). The reaction mechanism and pathways of supercritical transesterification were not described (Anitescu and Bruno, 2012), and a future research in understand it is necessary.

The flow system is better suited for scale-up or scale-out over the batch. The flow reactor would be able to continuously produce FAEE without the need to cool the reactor between batches, and therefore, should demand less energy and footprint. In spite of these advantages, supercritical fluid technology still has some obstacles for its implementation, such as the cost associated with the equipment, energy demand and also safety issues (Tan and Lee, 2011, Kim et al., 2013).

The high energy demand could be alleviated somewhat by the integration of the heating and cooling system (Glisic and Skala, 2009) that may result in a similar energy demand to that of the catalyst transesterification method (Tan and Lee, 2011). An energy analysis based on the route proposed in this work is discussed in the next chapter.

The cost of the plant includes the cost of equipment to withstand high temperature and pressure conditions, pumps, and cooling units. There are also costs associated with the OPEX, energy consumption and maintenance. The cost challenge in supercritical transesterification with vegetal oil has been assessed previously in the literature, and the results indicated that the price could be competitive to the catalysed process (van Kasteren and Nisworo, 2007).

The high temperature and pressure in the SCDT will require a safety management policy of the system at large scale. There is no detailed studies of this topic due to the lack of existing large scale plant and further assessment needs to be done (Silva and Oliveira, 2014), however there are numerous

commercial processes operating at similar or higher conditions; there is also the consideration of scale out rather than scale-up.

5.3.9.2. Product Analysis

Another difference from the batch reactor studies was the composition of the FAEE product. The results are presented in Table 5.8.

Table 5.8. FAEE profile of *C. vulgaris* when used catalyst transesterification and SCDT in batch and flow reactors (average value from the conditions described in Chapter 5)

FAEE (mass %)			CT	SCDT (Batch)	SCDT (Flow)
C16:0	$C_{18}H_{36}O_2$	Ethyl Palmitate	16.7	65.4	17.7
C18:0	$C_{20}H_{40}O_2$	Ethyl Stearate	0.3	3.1	0.9
C18:1	$C_{20}H_{38}O_2$	Ethyl Oleate	3.0	4.1	2.9
C18:2	$C_{20}H_{36}O_2$	Ethyl Linoleate	18.1	8.7	18.7
C18:3	$C_{20}H_{34}O_2$	Ethyl Linolenate	62.0	18.7	59.9

These ratios suggest that during the batch reaction, with its long exposure to high temperature, there is trans-isomerization and decomposition of the more unsaturated fatty acids, such as linolenic acid occurred. This effect reduces fuel stability and cold flow properties (Imahara et al., 2008) and hence represents poorer fuel quality (Silva and Oliveira, 2014). This decomposition has been shown elsewhere by Olivares-Carrillo and Quesada-Medina (2011) who demonstrated thermal degradation at 300°C after 75 min reaction and by Levine et al. (2010). The latter concluded that higher temperature and longer retention times cause greater trans-isomerization of linoleic and linolenic acid ethyl ester as well as thermal reactions of unsaturated FAEE to heavier unidentified compounds (Imahara et al., 2008).

The increase in linolenic acid ethyl ester in the flow reactor compared to the batch reactor shows another advantage of this system that is it decreases the decomposition of unsaturated FFA.

5.3.10. Char Analysis

The remaining material in the reactor was also analysed. The samples were analysed for CNH content and by SEM.

Table 5.9 shows the elemental analysis. The results indicate a larger reduction on carbon content in the SC-EtOH reaction than in the CT, but this value increases if CO_2 is used as co-solvent probably from carbon enrichment of the char with its addition. This was also revealed before by Levine et al. (2010) who showed the carbon amount increased from 38% to 62% in *Dunaliella salina*.

Table 5.9. Elemental analysis of the char (mass %) as described in section 4.2.4

	C	N	H
Dry Algae	47.2	8.0	7.0
SC-EtOH	30.1	2.9	3.2
SC-EtOH – adding CO_2	67.2	4.7	4.14
CT	40.7	6.5	7.65

*SC-EtOH with 100 mg algae to 5 mL of water and 50 of mL ethanol at 300 rpm, 270°C for 15 min
*CT with sulphuric acid (5% w/v in ethanol) after soxhlet extraction with hexane

SEM was used to analyse the degradation of the biomass after the exposure to supercritical ethanol. Figure 5.19 shows the ash obtained by the methodology described in section 4.2.7 and char after processing algae in the batch and flow reactors.

After the reaction with supercritical ethanol, it is possible to notice some smooth particles that are not visible in the ash (red circles in Figure 5.19). According to Senneca (2011), these particles are rich in carbon and indicates presence of organic components from incomplete reaction (Hu et al., 2013). Samples recovered from the flow reactor also showed some hydrochar similar to the one produced by the hydrolysis of cellulose chains that takes place between 210°C and 220°C (Sevilla and Fuertes, 2009).

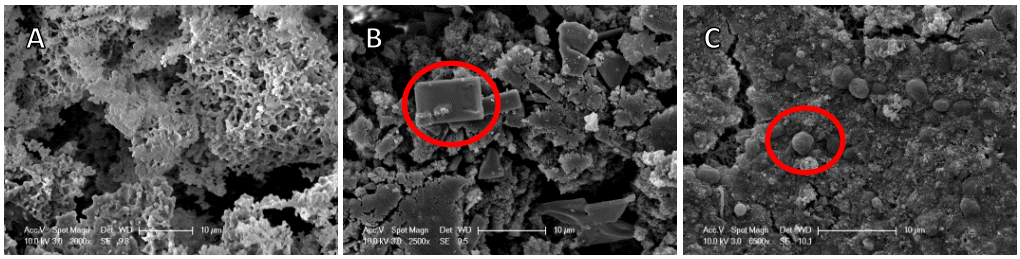

Figure 5.19. SEM images with smooth particles highlighted by red circles A) Ash biomass obtained from methodology described in section 4.2.7; B) Char from batch reactor (270°C, 15 min, 300 rpm, 100; 100 mg algae to 5 mL of water and 50 of mL ethanol); C) Char from flow reactor (260°C, 70 bar, 20 min, 10 mg algae per mL of water)

The analysis of the char was important to show that the process did not consume all the volatile matter and there is still some energy available in the solid residue even after the transesterification and shows the potential of this feedstock to the biorefineries concept. The char obtained from the SCDT (especially the one with high carbon content) could be used as fuel or for soil amendment in order to enhance the carbon amount, microbial activity and fertilizer action (Steinbeiss et al., 2009).

5.4. Conclusions

Direct transesterification of *Chlorella vulgaris* in supercritical ethanol was proposed and tested. The aim of using the algae direct from harvesting after only passing through a dewatering step to leave a high water content was accomplished, but the process still gave a low efficiency achieving a maximum yield of 47.5% wt. at 260°C, around 75 bar, 2 $mL \cdot min^{-1}$ in the flow reactor with algae concentration in water of 6 $mg \cdot mL^{-1}$.

Preliminary tests were done in a batch reactor where it was found that a large amount of alcohol solvent was required due the water content of the algae (50 mL ethanol to 100 mg algae in 5 mL water). The supercritical point of this mixture was calculated to be higher than that of the pure ethanol (255°C and 74 bar). The influence of the water content and temperature was also established. The water is important to the hydrolysis of TG to FFA, but in high amounts (>45 mL per g of algae) it begins to degrade the FAEE. A dewatering step is therefore still necessary for the algae transesterification, in this case this was achieved by centrifugation (from 1 $mg \cdot mL^{-1}$ as harvested to 20 $mg \cdot mL^{-1}$ after dewatering). Increasing the temperature gave an increase in FAEE yield, but after long retention time (4 hours) lead to increased degradation of FAEE; a temperature of around 270°C is preferred.

Experiments with TG and FFA achieved their maximum conversion after 30 minutes (according to the literature), however algae only had a maximum yield in the batch reactor (26% wt.) after 6 hours of reaction. This was assumed to be

from the presence of the cell wall and other components within the *Chlorella vulgaris* that hindered the reaction.

Investigations into increasing the yield were conducted and some solutions were proposed. The first was to add a catalyst ($ZnAl_2O_4$) to improve the reaction rate, but the presence of water inhibited its action. The second was to use a co-solvent in order to change the solubility of the mixture. The use of CO_2 improved the yield and it is recommended to use it in the flow reactor.

In the flow system, pressure and retention time were import to the FAEE yield, but it was not possible to find the best conditions due the instability of the flow control and pressure in the system. The flow reactor improved the yield in comparison to the batch reactor with a lower retention time (36% wt. FAEE in 20 minutes compared to 26% in 6 hours) and gave a lower degradation of unsaturated FA. The flow process with the algae as direct reactant had some problems of design that were not able to be solved during this PhD and need to be addressed in future research. The use of pump and valves able to cope with suspended solids while delivering very high pressures is a necessary improvement. Thereby, flow and pressure could be better controlled and a proper DoE could be done to find the best conditions for system operation.

Other significant finding of this study was the large presence of phytol in the biodiesel product that could be further explored and studied as a fuel additive. The char analysis also demonstrated the potential for investigating algae as a feedstock for biorefineries.

6. Environmental Analysis and Energy Balance of the Process

6.1. Introduction

The final part of this thesis is to analyse the environmental and energy feasibility of the new proposed route to produce biodiesel from algae using supercritical ethanol. The energy balance is necessary in order to compare the traditional catalyst route with the supercritical route to check if there is any advantage. The new method eliminates the use of catalyst and drying but requires an increase in the reaction temperature and pressure during the transesterification.

An environmental analysis is also necessary to enable a complete evaluation of the new route to biodiesel production. GaBi software was chosen to make this analysis consistent with other published studies and to help consider all steps of the process.

There are several studies comparing different algae upstream routes (Jorquera et al., 2010). The objective of this study is to focus on the downstream processes after the cultivation and dewatering that are considered the same for both the methods studied.

The catalyst route includes drying the biomass, extracting the lipids and their conversion to FAEE via transesterification with catalyst. The second route

includes the transesterification without the use of a catalyst at high temperature and pressure in supercritical ethanol (SC-EtOH) where the phases of drying and extracting the lipids are eliminated.

These two routes were compared and the industrial soybean biodiesel route was used to evaluate the benefits of the SC-EtOH to that of the commercial feedstock.

The baseline process was selected from the literature as follows and described in Figure 6.1. Open ponds to cultivate the algae (Stephenson et al., 2010), harvesting through flocculation with aluminium coagulant and dewatering by centrifugation. This is followed by drying by rotary press, oil extraction with hexane and transesterification with an acid catalyst. The SC-EtOH uses a reactor where the biomass after dewatering is pumped with ethanol into a flow reactor where the transesterification happens in the supercritical phase at a temperature and pressure above 240.9°C and 61.4 bars respectively (Figure 6.2).

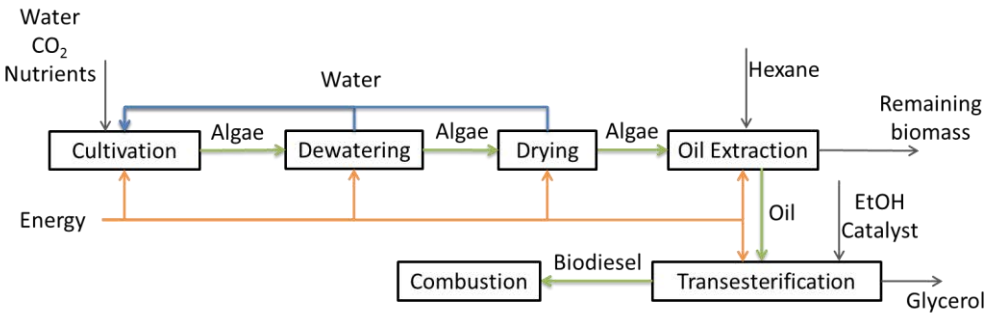

Figure 6.1. Traditional catalyst route diagram

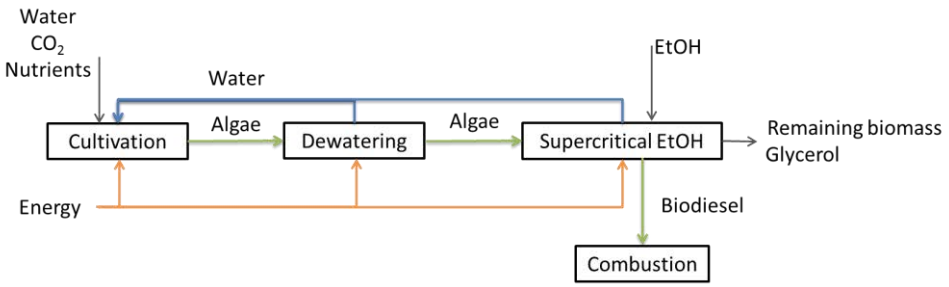

Figure 6.2. Supercritical EtOH route

6.2. Methodology

The process analysis was done based on two main parameters: Net Energy Ratio (NER) and environmental impact. Based on the LCA methodology, the steps of scope definition, inventory analysis, assessment and then interpretation were followed.

In order to standardise the comparison, a functional unit of 1 kg of biodiesel with energy of 37.8 $MJ \cdot kg^{-1}$ and a system boundary of "well-to-gate," which includes the cultivation, harvesting, lipid extraction, and oil conversion to biodiesel were adopted. The amount of algae produced was adjusted to the production of the functional unit depending on the efficiency of the conversion method. All the energy and impacts calculated in the process were allocated to the produced biodiesel, but it is important to note that there is cogeneration of other products such as glycerol and residual biomass that could also have its own market, but are not considered on this analysis.

The net energy ratio (NER) was calculated by the ratio between the energy output generated and the energy input to the system as shown by the equation below:

$$NER = \frac{Energy\ output}{Energy\ Input}$$ **Equation 6.1**

When the NER is bigger than one, the process is energetically viable and if it is less than one it indicates that the process requires more energy than it produces.

The environmental analysis was done using GaBi LCA software. It was decided to use the CML 2001 (Centrum Milieukunde Leiden) method that includes the following factors (Acero et al., 2014):

- Abiotic Depletion (ADP elements and ADP fossil)
  - The use of resources is measured in kg Sb (antimony) equivalent for elements and MJ for fossil fuels.
- Acidification Potential (AP)
  - The measurement of the acidifying effect from the emission presented in kg SO_2equivalent.
- Ecotoxicity Potential (Freshwater Aquatic, Marine Aquatic, and Terrestrial)
  - A method to measure the toxic substances' effects on the ecosystem, separated in freshwater, marine and terrestrial in kg DCB (dichlorobenzene) equivalent.
- Eutrophication Potential (EP)
  - The accumulation of nutrients in an ecosystem which causes an excessive biomass growth (such as algae) measured in kg PO_4^{3-}(phosphate) equivalent.
- Global Warming Potential (GWP 100 years)
  - The climate change representation in kg CO_2 equivalent based on the emission of greenhouse gases.
- Human Toxicity Potential (HTP inf.)

    - An index to represent the potential harm of chemicals in the human body based on the toxicity and dose of the compound and expressed in kg DCB equivalent.
- Ozone Layer Depletion Potential (ODP)
    - The measurement of the damage of the ozone layer by anthropogenic emissions represented in kg CFC-11 equivalent.
- Photochemical Ozone Creation Potential (POCP)
    - Increase of the "ground level ozone" by emission of CO, SO_2, NO, ammonium and NMVOC (non-methane volatile organic compounds) generating the summer smog that is toxic to humans, measured in kg Ethene-Equiv.

CML 2001 is a method developed by the Institute of Environmental Sciences – Leiden University. It limits the modelling to the early stages in the chain to decrease the uncertainties (GaBi, 2016). For each of the design processes, and their inputs and outputs, the software using its database is able to generate a report with the environmental impacts values calculated directly by this methodology.

The software also brings the possibility of selecting the energy grid location. Based on the analysis on section 3.3.3, Brazil was selected as location and base energy grid input. When it is not available the Brazilian location for some parameters this is gotten from Global database.

In order to close the analysis about the viability of the new supercritical fluid route to produce a third generation biodiesel, it was decided to compare its impacts and energy balances to the first generation biodiesel already established

in the market: biodiesel from soy oil. The values of energy and environmental impacts for soy biodiesel were collected from the literature and compared to the ones found for the algae biodiesel routes, trying to maintain the same assumptions during the calculations.

6.3. Results and discussion

6.3.1. Scope definition

The energy analysis was defined for the production of 1 kg of biodiesel with 37.8 MJ of energy (low heating value), from algae cultivation to biodiesel production (well-to-gate) as shown in the figure below.

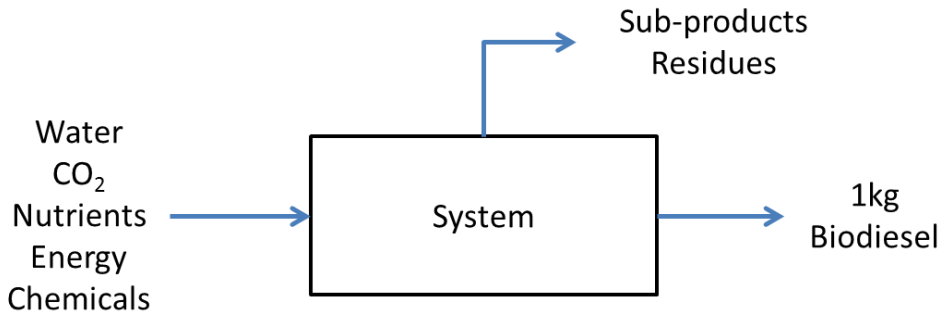

Figure 6.3. Scope definition (boundary limited by the system)

Sub-products (such as glycerol), destination of residues and the source of inputs to algae cultivation were not specified in the scope and are outside of the boundaries of the analysed system. The material, costs and energy for facilities constructions were not considered. The environmental analysis also includes the combustion of the biodiesel, since it is important to include the emissions during this is stage in the balance of CO_2-eq.

All the impacts and energy used were allocated to the biodiesel, so the co-products were not considered as having an effect on the production chain. With a stablished market for the products the total impact of the process chain could be divided by them, but in this case the process has been considered only for the biodiesel market.

6.3.2. Inventory analysis

6.3.2.1. Algae strain

The algae selected for the study was *Chlorella vulgaris*. The data for the LCA were taken from previous literature, especially the work from Lardon et al. (2009), Stephenson et al. (2010) and Collet et al. (2014a) that used the same strain.

In this work, this strain was cultivated in controlled environment at University of Birmingham with a production of 0.4 kg algae $\cdot m^{-3}$ of media and 7.28% wt. oil as FAEE. It is worth noting that previous work already achieved more than four times this production (Stephenson et al., 2010) with an oil content close to 50% wt.

It is important to state that the algae cultivated in the lab at UoB for the LCA, was not considered since the *Chlorella vulgaris* used in this project showed lower specifications in terms of lipid and biodiesel yield when compared to the same specie cultivated under nitrogen stress and in raceway open pond (Chisti, 2007). In order to have the most realistic scenario, the production considered was

that achieved in UK of 1.67 kg·m^{-3} (Stephenson et al., 2010) and an oil content of 20% wt. This, however, is still pessimistic when compared to other works, but is more realistic at large scale due to problems during scale-up, such as cloudy periods, plagues and other growth problems.

6.3.2.2. Cultivation – Raceway open pond

The open pond technology was selected as it is a more developed technology and easier to scale-up. In comparison, there is diverse research in lab-scale photobioreactors, but when they are built at larger scale there are issues in maintenance of stable conditions (Brentner et al., 2011). Open pond designs were studied by Chiaramonti et al. (2013) and Liffman et al. (2013) and the design parameters adopted in this work are presented in Table 6.1 (Lardon et al., 2009).

Table 6.1. Open pond design

Parameters	Raceway Open Pond	Unit
Volume	480	m^3
Wide	10	M
Long	100	M
Deep	0.3	M
Hydraulic depth	0.25	M
Velocity	0.25	$m \cdot s^{-1}$
Productivity	25	$g \cdot m^{-2} \cdot day^{-1}$
Concentration	1.67	$kg \cdot m^{-3}$
Days of operation	300	Days

Based on this design, the energy consumption used for the circulation of the pond can be estimated by the equation (Razon and Tan, 2011):

$$\boldsymbol{E = \frac{\rho Q \Delta d}{0.102 e}\, t} \qquad \textbf{Equation 6.2}$$

Where, E = energy (kWh), t = time (h), ρ = density of water ($kg \cdot m^{-3}$), Q = water flow rate ($m^3 \cdot s^{-1}$), e = paddle wheel efficiency and Δd = head loss (m). The efficiency and head loss were taken from literature as common values for open ponds (Δd = 0.06 m and e = 0.17) (Razon and Tan, 2011) and flow rate and time were assumed as presented in Table 6.1. The energy calculated for the pond is approximately 0.2 $kWh \cdot kg^{-1}$ algae which matches values used in the literature (Ventura et al., 2013, Collet et al., 2014a).

Besides the energy of the paddlewheels, there is also energy consumed during the pumping of CO_2 and nutrients. This was assumed to be 0.23 $kWh \cdot kg^{-1}$algae (Ventura et al., 2013); so the total energy requirement in the cultivation phase for this study was 0.43 $kWh \cdot kg^{-1}$ algae.

Some inputs required for the cultivation stage are presented in Table 6.2. These data were used in GaBi to calculate the environmental impact.

Table 6.2. Inputs during cultivation

Parameters	Quantity	Unit	Reference
Fixed CO_2	1.8	kg $CO_2 \cdot kg^{-1}$	(Patil et al., 2011)
Nutrients			(Lardon et al., 2009)
• Nitrogen	0.06	kg	
• Phosphorus	0.008	kg	
• Potassium	0.007	kg	
Water (losses)	4	litres	(Lardon et al., 2009)
Energy	0.43	$kWh \cdot kg^{-1}$algae	(Ventura et al., 2013)

The cultivation step was considered the same for both cases, the base line and the SC-EtOH route, and was utilized to allow a comparison against biodiesel produced from the first generation approach.

6.3.2.3. Harvesting and dewatering

The harvesting and dewatering are made in a series of steps. First bulk harvesting separates the main slurry which is usually done by flocculation using ferric chloride ($FeCl_3$), aluminium sulphate ($Al_2(SO_4)_3$) or ferric sulphate ($Fe_2(SO_4)_3$). After this step, the slurry is concentrated by thickening using centrifuge, filter or ultrasonic aggregation. The algae are then dried, in the catalyst route, either by a drum drying, freeze-drying, solar drying or a rotary press.

In this study an aluminium coagulant, followed by centrifugation and a drying by rotary press was used. This route was selected as it was assumed to be the easiest to scale. The drying step is eliminated in the supercritical route as wet biomass with high water content can be used. The main assumed data are presented in Table 6.3.

Table 6.3. Harvesting, dewatering and drying parameters

Parameters	Quantity	Unit	Reference
Alum coagulant	0.74	$g \cdot m^{-3}$	(Ventura et al., 2013)
Efficiency of the separation	95	%	(Collet et al., 2014a)
Energy Flocculation	0.025	$kWh \cdot kg^{-1}$ algae	(Stephenson et al., 2010)
Energy Thickening	0.027	$kWh \cdot kg^{-1}$ algae	(Stephenson et al., 2010)
Concentration after dewatering	50	kg algae$\cdot m^{-3}$	(Collet et al., 2014a)
Energy Drying	0.1	$kWh \cdot kg^{-1}$ algae	(Brentner et al., 2011)
Concentration after drying	200	kg algae$\cdot m^{-3}$	(Lardon et al., 2009)

6.3.2.4. Oil extraction and transesterification

6.3.2.4.1. Catalyst route

In order to extract the oil, the algae have to first pass through a cell disruption method. This process can be energy intensive depending of the chosen technology. In this study, it was decided to assume the most common technology applied with a consumption of 0.136 $kWh \cdot kg^{-1}$ as suggested by Brentner et al. (2011) for the entire extraction process including sonication, filter press, and the energy used to maintain the pressure and temperature during the reaction and for recovering the solvent. Bead milling, for example, is also a technology cited in the literature (Günerken et al., 2015).

The oil is assumed to be extracted with a solvent comprising hexane and ethanol in amounts of 7.92 kg hexane and 0.88 kg ethanol per kg biomass with an extraction efficiency of 90%. The oil is then transesterified with the addition of a catalyst.

According to the research, acid transesterification is the preferred option (as demonstrated in Figure 4.19, section 4.3.4.2), so H_2SO_4 was selected as the catalyst. The production assumes all the extracted to be converted. The energy needed for the transesterification was assumed to be 0.12 $kWh \cdot kg^{-1}$ oil, considering the mixing electricity and heating (Ventura et al., 2013). In the catalyst route, the biodiesel also needs to be subjected to a refining process to remove the catalyst and the excess solvent. This step would require an additional energy input of 0.19 $kWh \cdot kg^{-1}$ biodiesel (Stephenson et al., 2010).

6.3.2.4.2. Supercritical EtOH

The necessary energy in the route with supercritical ethanol is that required by the pump and the heat provided by the oven. These two demands of energy were the only ones considered; some energy will also be required in the cooling process and solvent and water recovery. These values were neglected because of its magnitude, the cooling process works reusing energy of the heating oven (by heat exchanger or regenerator) and the separation process is mainly done by gravity.

According to Shimako et al. (2016), the electricity required by the pump can be calculate from a derivation of Bernoulli (considering no charge losses, no changes in fluid velocity, no elevation and a pump efficiency of 80%):

$$E = \left(\frac{m_{EtOH}}{m_{biodiesel}}\right) \frac{\Delta P \ 10^{-6}}{0.8 \ \rho_{EtOH}} \qquad \textbf{Equation 6.3}$$

Where E is electricity (MJ / kg biodiesel), m_{EtOH} is mass of ethanol, $m_{biodiesel}$ is mass of biodiesel, ΔP is pressure change (70 bar) and ρ_{EtOH} is density of the fluid (789 $kg \cdot m^{-3}$ at room temperature). The mass ratio between ethanol and biodiesel can vary according to the assumptions. Following the experiments carried out in Chapter 5 (5 mL of ethanol to each 100 mg of algae) the energy required would be 3.04 $kWh \cdot kg^{-1}$ biodiesel which would make the route non-viable. If the molar ratio proposed by the literature (1:36 oil:ethanol) is adopted, the energy used would be 0.05 $kWh \cdot kg^{-1}$ biodiesel produced for 40% yield and 0.03 $kWh \cdot kg^{-1}$ for 60% yield. By the biodiesel production reaction each mol of biodiesel produced would require one mol of ethanol, so 35 moles would be not used in the process

and could be recirculated after the separation from the water, per example, by distillation which would require more 0.06 kWh·kg^{-1} ethanol (Gil et al., 2008).

The heat provided by the oven can be calculated by:

$$Q = m\ C\ \Delta T \qquad \textbf{Equation 6.4}$$

Where Q is the heat, m is the mass, C is the specific heat and ΔT is the change in temperature. In this case it was considered the mass of ethanol used to produce 1kg of biodiesel (functional unit), C of 6.55 kJ·kg^{-1}·K^{-1}, temperature change of 260°C, and an efficiency of 80% was assumed, which resulted in an energy requirement of 0.93 kWh·kg^{-1} biodiesel for the process with 40% yield and 0.59 kWh·kg^{-1} biodiesel in the process with 60% yield.

An important highlight of the SCDT is the necessary adaptions on it in order to make the process viable, the high ethanol proportions to algae biomass needs to be decreased or the pump energy would be greater than that from the biodiesel.

The supercritical route has the advantage in not requiring the separation of the catalyst as it is not used and the solvent, glycerol and water separation from biodiesel is done through gravity so the energy consumption can be despised. The total required energy in these process is 1.07 kWh·kg^{-1} for 40% yield and 0.68 kWh·kg^{-1} for 60% yield. These values calculated here are similar to the ones presented in the literature (Glisic and Skala, 2009, Brentner et al., 2011).

6.3.2.5. Products and combustion

During the process biodiesel and glycerol are produced, in addition, there are solvents that may be reused and also some remaining biomass that can be anaerobic digested to raise more energy. The heat energy used in the transesterification also can be reused. In this balance, only the energy from the biodiesel has been considered in order to allow a comparison of this product. It is important to highlight that the potential of algae for energy production can be greater if energy recycling is adopted and the remaining biomass is also considered.

Biodiesel from algae was assumed to have the same energy content as that of biodiesel produced from other vegetal oils with an amount of 37.8 $MJ \cdot kg^{-1}$ of fuel. Its combustion was considered for cargo transportation in order to undertake the environmental analysis.

6.3.3. Energy analysis

Net energy analyses have been previously reported in the literature. Razon and Tan (2011) stated that there are uncertainties around the energy balance calculation, mainly because there is no facility in large scale for algae cultivation so the energy consumption and losses are estimated. This work makes an energy balance in order to compare the downstream process (oil extraction and transesterification) and evaluating the new route proposed. The energy values detailed above in section 6.3.2 were converted to the functional unit ($kWh \cdot kg^{-1}$ biodiesel) and are presented in Table 6.4.

Table 6.4. Net energy ratio calculation

Energy Balance (kWh·kg^{-1} biodiesel)			
	Catalyst Route	**Supercritical EtOH (40%)***	**Supercritical EtOH (60%)***
Cultivation	2.79	5.66	3.77
Harvesting / Dewatering	0.32	0.66	0.44
Drying	0.62	-	-
Extraction	0.86	-	-
Transesterification	0.13	1.07	0.68
Refining	0.19	-	-
Total	**4.91**	**7.39**	**4.89**
Biodiesel energy	10.50	10.50	10.50
Net Energy Ratio	**2.14**	**1.42**	**2.15**

*The percentage (40% and 60%) represents the efficiency of the SC-EtOH transesterification route

The results show that the catalyst route and the supercritical route have energy ratios above one, and are therefore both energetically viable. This ratio could be even larger if the biomass remaining afterwards is used to generate energy (the biomass has 60% wt. of volatile matter and 15% wt. of fixed carbon that could be used in future energy generation in gasifiers and as char respectively).

The SC-EtOH with 40% yield has a worse net energy ratio than the catalyst route, however if the efficiency of the transesterification increases from 40% (the maximum achieved during the experiments presented here) to 60% this route would be better than the traditional one (as showed in Table 6.4), demonstrating the potential of this technology. This study was not able to achieve it but it is an attainable number compared to other oil transesterification in supercritical ethanol environment. In order to achieve the necessary efficiency, the process

need to be improved changing some parameters such as increasing temperature or retention time or even using a pre-heating step.

The results show that after the cultivation phase the extraction step is the most energy consuming. Its elimination, even with the increase in energy for the transesterification would be beneficial as a way to reduce overall energy use.

6.3.4. GaBi Software

The GaBi software was used to calculate the environmental impacts, the inventory described in section 6.3.2 was included in the system in the Brazilian location, where it is intended to install the facility. The following flowcharts were created using the software:

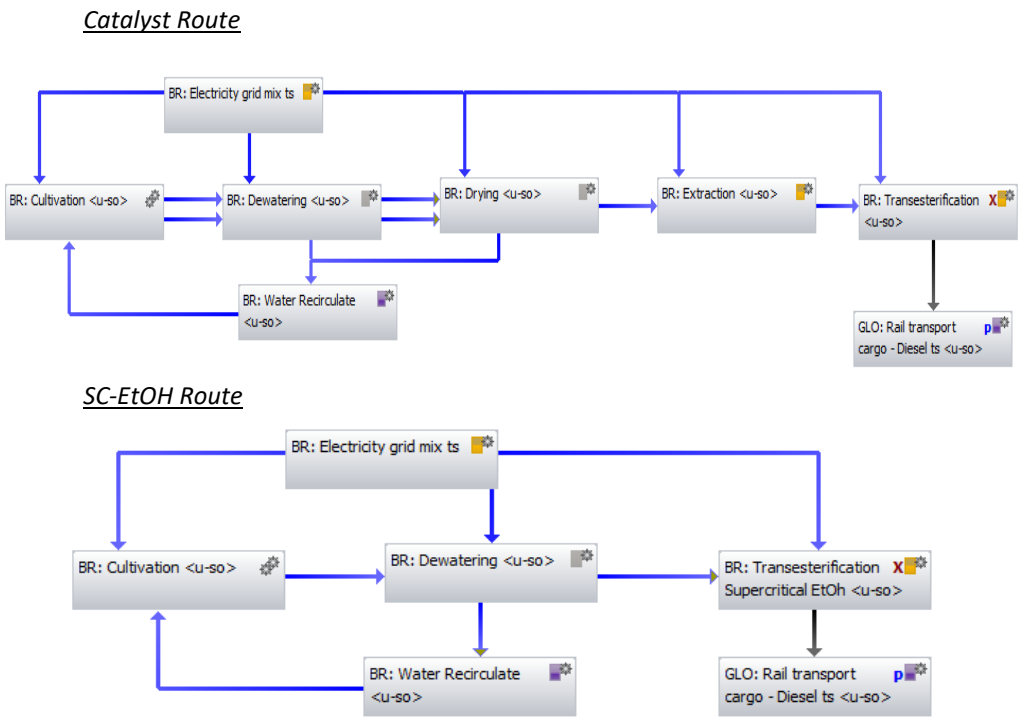

Figure 6.4. Flowcharts imported from GaBi showing the routes for biodiesel production

At this stage, it is important to discuss the considerations from the LCA Software. The energy input to the system was considered from the grid mix for Brazil (BR), which has a degree of uncertainty. The environmental impacts in this location are also smaller than in the UK or Europe since the electric grid is based on hydropower and is therefore more sustainable. When it is not available, the standard global process was used (GLO). The plant is assumed to be installed close to CO_2 generators and may use integration of energy from the biodiesel plant and nearby industry.

Another assumption is the direct combustion of the biodiesel in cargo transportation. A more complete LCA would also include transportation to the pump, but as the objective of this project is only to compare the process routes, this step was ignored in order to decrease the errors of assumptions.

The water removed from the algae in the dewatering and drying process was recirculated to the cultivation stage. This step is important to reduce the water consumed and consequently the environmental impact. The water and ethanol used in the SC-EtOH transesterification were not recirculated (as this procedure was not tested in the experiments), but a distillation step or even the use of membranes could make it possible in future system improvements. In this model, the water and the not used ethanol were only separated from the biodiesel and discarded not recirculating or being allocated.

The materials of construction for both facilities were not considered in this project due to the fact there is no large scale plant in the market for both routes

and therefore any assumptions would increase even more the errors associated with the LCA. A complete assessment is necessary in the future in order to ascertain the lifespan of the open ponds and associated equipment (such as pumps, tubing and reactors) and to measure the impacts of the plant installation and work.

6.3.5. Environmental impacts

Table 6.5. Environmental parameters from GaBi per kg of biodiesel

Environmental Parameter	Unit	Traditional Route	Supercritical EtOH (40%)	Supercritical EtOH (60%)
Abiotic Depletion (ADP elements)	kg Sb-Equiv.	0.00	0.00	0.00
Abiotic Depletion (ADP fossil)	MJ	46.69	67.54	45.23
Acidification Potential (AP)	kg SO_2-Equiv.	0.18	0.19	0.18
Eutrophication Potential (EP)	kg Phosphate-Equiv.	0.04	0.05	0.04
Freshwater Aquatic Ecotoxicity Pot. (FAETP)	kg DCB-Equiv.	0.11	0.15	0.10
Global Warming Potential (GWP 100 years)	kg CO_2-Equiv.	-52.09	-154.93	-93.83
Human Toxicity Potential (HTP inf.)	kg DCB-Equiv.	1.90	2.58	1.86
Marine Aquatic Ecotoxicity Pot. (MAETP)	kg DCB-Equiv.	1592.02	2303.15	1542.25
Ozone Layer Depletion Potential (ODP)	kg R11-Equiv.	0.00	0.00	0.00
Photochem. Ozone Creation Potential (POCP)	kg Ethene-Equiv.	0.02	0.03	0.02
Terrestric Ecotoxicity Potential (TETP)	kg DCB-Equiv.	0.04	0.05	0.04

The environmental impacts were quantified and are presented in Table 6.5. In general, the impact of SC-EtOH route on the efficiency achieved in the laboratory is environmentally worse than the traditional route of catalyst transesterification, but as soon as the transesterification efficiency increases to 60% the values are similar. Specifically, most of the impact comes from the electricity used, combustion of the diesel and the algae cultivation. Two examples showing the sources of environmental impacts are presented below.

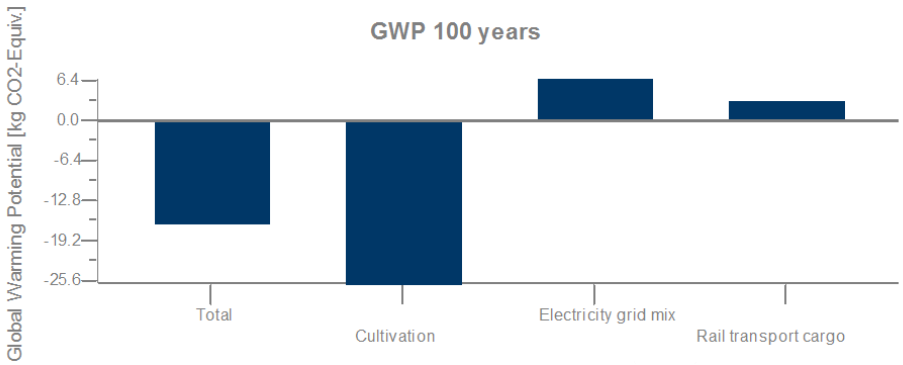

Figure 6.5. Global warming potential (GWP) for SC-EtOH route 40% conversion

The Global warming potential (GWP) is negative in all the cases as the capture of CO_2 and GHG is larger than the emissions generated from the combustion and therefore has a positive climate change impact hence demonstrating the benefits of crude oil diesel replacement by biodiesel. One surprising result is that GWP is better in the SC-EtOH route with 40% efficiency. This can be explained by the input selection during the LCA construction. The CO_2 is captured in the cultivation phase, so as a greater amount of algae is needed to produce the same amount of biodiesel the CO_2 used is greater, and consequently the GWP is smaller (better). GaBi calculations of the GWP show some important

considerations about the LCA methodology, GaBi considers the capture of the CO_2 by the algae but does not predict that some of it can come back to the environment in the remaining biomass, so it is considered a weakness in the database.

The other environmental factors are worse in SC-EtOH route with 40% yield, because more nutrients are necessary during cultivation as well as more energy for the algae processing.

The Marine Aquatic Ecotoxicity Potential values were extreme in all the cases achieving up to 2303 kg DCB-Equiv per kg of biodiesel, which equates to 330 kg DCB-Equiv per kWh. According to the balance, this results from the energy grid chosen and specifically calculated for the metal depositions. The CML methodology assumes an emission of $1.17 \cdot 10^{-05}$ kg of hydrogen fluoride and $3 \cdot 10^{-5}$ kg of vanadium emissions per kWh from the power plants and CML method considers these two substances 41 million and 12 million times more toxic than 1.4 DCB that is the characterizing substance. This demonstrates the complexity of toxicity data and the challenges of condensing such data to a single index. It is necessary to include a better evaluation of the input electricity source when deciding where to locate the algal biodiesel plant.

Another interesting result is the abiotic depletion by fossil fuels which shows a use of more than 45 MJ. This is more energy than is produced by the biodiesel. If the energy associated to the inputs (such as ethanol used as solvent, CO_2 during the cultivation) and the energy grid for the electricity used are

considered the biodiesel from algae would not be advantageous for production, so a further analysis is necessary to balance the energy in the inputs and how much of it is reused.

This contradicts the energy balance done previously in section 6.3.3. The NER was calculated with the energy used to operate the facility and not the energy associated to the inputs such as the fertilizers and solvents. The process contains remaining energy not considered in this balance in form of solvent (39 mols of ethanol to each mol of produced biodiesel) and biomass (only 20% wt. of algae is oil used in this process). This emphasises the importance of the solvent route (especially ethanol), nutrient recycling, and biomass exploitation to make the fuel production viable.

6.3.6. Comparison with other feedstock

The soybean biodiesel route was selected to compare with the algae biodiesel route because of its established market, especially in Brazil (Stattman et al., 2013). It also has similar oil content, around 20% wt. (Lee et al., 2013, Hammond et al., 2005, Breene et al., 1988).

The soybean biodiesel production process can be described in parallel to the algae one, with the phases of cultivation, harvesting, oil extraction and conversion to biodiesel. In this study, the same methods for the oil extraction from soybean and algae and conversion of oil are considered. The main differences between algae and soybean biodiesel are the upstream process which includes cultivation, harvesting and biomass drying.

The market for soybean is more structured than the algae and because of its cultivation for other applications (food) its production is already done on a large scale. The cultivation of soy includes the impacts of fertilizers and in land use change, and as result the environmental impacts are larger than those of algae biodiesel. Soybean requires the application of nitrogen, phosphorus, potassium, magnesium and other nutrients direct to the soil (Niederl and Narodoslawsky, 2004).

Some studies have assumed the environmental impacts from the cultivation of soy greater than all algae biodiesel production route. Panichelli et al. (2009) stated a GWP of 3.5 kg CO_2-eq per kg of soybean biodiesel for a farm in Argentina. Algae biodiesel might be a better environmental option due to the amount of CO_2 captured per kg of biodiesel produced during its cultivation and the lower amount of nutrients (from fertilisers) used when compared to field crops. This is also similar for the other impacts parameters where soybean biodiesel is worse than the algae one; these are discussed below.

According to Pradhan et al. (2011), the cultivation of soybean would require 1.89 kWh·kg^{-1} of biodiesel produced with no allocation to other products and only 0.32 kWh·kg^{-1} if the soybean meal was considered as a coproduct (with mass-based allocation). If all the upstream processes are included then the energy to crush this feedstock would be 3.89 kWh·kg^{-1} biodiesel, which is less than the algae demand. Using the literature energy consumption for soybean cultivation and oil extraction (1.89 kWh·kg^{-1} and 2 kWh·kg^{-1}) added to the same downstream

that the algae (transesterification and refining 0.32 $kWh \cdot kg^{-1}$). This study assumes no allocation to by-products.

Table 6.6 shows the total energy requirements for the soybean and algae routes.

Table 6.6. Comparison of energy demand from the different routes

Route	Energy ($kWh \cdot kg^{-1}$biodiesel)	Net Energy Ratio
Soybean	4.21	2.50
Algae - Traditional Route	4.91	2.14
Supercritical EtOH (40%)	7.39	1.42
Supercritical EtOH (60%)	4.89	2.15

The energy balance shows that soybean biodiesel has more advantages over algae biodiesel, even when the efficiency is 60%. It demonstrates that the energy consumption during the downstream process is not the key value to make the algae biodiesel route feasible compared to biodiesel from soybean, but that the cultivation and harvesting steps need further research in order to decrease their energy demand.

Methods of decreasing the energy requirement in the cultivation step would be to either make genetic modifications to the strain, changing the cultivation conditions in order to increase the oil content, or developing methods to increase the efficiency of the ponds.

This energy difference can become larger if the allocation to coproducts is applied. Soybean meal has already a structured market and it is therefore

important to create a market for the algae coproducts in order to decrease the impacts and demands to fuel.

An additional method to make biodiesel from algae more feasible is to use the algae to capture CO_2 or treat wastewater. In this way, processes that are usually costly and energy demanding would be done in conjunction with biodiesel production.

6.4. Conclusions

This chapter shows a life cycle analysis of the new route proposed to produce biodiesel from algae using supercritical ethanol which eliminates the use of catalyst and the algae drying process. An energy analysis (through net energy ratio) and an environmental analysis using the CML 2001 method (which includes abiotic depletion – elements and fossil; acidification potential; ecotoxicity potential – freshwater, marine and terrestrial; eutrophication potential; global warming potential (GWP); human toxicity potential; ozone layer depletion potential; photochemical ozone creation potential) were conducted. An energy comparison between the algae biodiesel and the biodiesel installed in the market (from soybean) was also conducted in order to assess the advantages of producing biodiesel from algae feedstock.

The SC-EtOH route when compared to the catalyst route (open pond cultivation, harvesting by flocculation, centrifugation and rotary press for drying, hexane extraction of the oil and transesterification with acid catalyst with an

efficiency of 90%) is only energetically feasible when the transesterification efficiency achieves 60% and with the ratio between ethanol and biodiesel decreased (around 1:36 molar ratio oil:ethanol). This represents a yield 50% greater than that achieved in the laboratory during this PhD. The Net Energy Ratio to produce 1 kg of biodiesel using the base route was 2.14, while with the SC-EtOH with 40% efficiency was 1.42 and with 60% efficiency was 2.15.

The environmental analysis agreed with the NER. The SC-EtOH route achieves similar environmental impacts to that of the base method when the efficiency was 60%. The GWP is the only environmental impact with opposite results, since it is highly influenced by the amount of algae cultivated; higher algae production is necessary when the transesterification efficiency is lower.

In comparison to soybean biodiesel, algae biodiesel presents environmental advantages mainly from the lower use of fertilisers to cultivate the crop, but performs lower in the energy balance. It highlights the importance of decreasing the energy requirements in the cultivation stage and creating a market for the co-products generated during the processes or combining biodiesel with other necessary processes, such as water treatment.

7. Conclusions and Future Work

7.1. Conclusions

The wet algae were found to be a possible feedstock for fuel production. This study showed that the three case studies (EU, US and Brazil) are able to grow the necessary amount of algae to produce biodiesel and meet the targets in their respective policies based on area requirements.

Considering the base-line productivity of 30,000 $L \cdot ha^{-1} \cdot year^{-1}$ for open pond facilities, the cultivation area requirement to achieve the *published targets* would be 9,000 km^2 for the EU, 18,900 km^2 for the US and for 2,300 km^2 for Brazil. For complete replacement of fossil-based diesel, the land demand would be 149,300 km^2 for the EU, 106,700 km^2 for the US, 29,700 km^2 for Brazil, and 811,600 km^2 for the world. It is important to highlight that these values only consider algae as a feedstock and for the real case there would also be an increase of other feedstocks and even some diesel replacement from other technologies. The objective was only to demonstrate the potential of biodiesel from algae.

The best locations for the diesel facilities would be the south of the EU, southeast of the US and along the Brazilian coast. These locations were based on the algae cultivation requirements: favourable climate (small temperature variation) and unoccupied flat land with close proximity to the necessary inputs (water, nutrients and carbon sources).

The use of algae for biodiesel production was shown to demand a lot of energy in the production process (around 4.91kWh·kg^{-1} biodiesel). The intention was to decrease the energy requirements of biodiesel production, by the use of downstream technology via direct transesterification in supercritical ethanol in order to eliminate the use of a catalyst and the drying step (the most energy demanding step in the process).

Chlorella vulgaris cultivated in BBM Media was selected and characterized in order to study this specific technology. The strain had in wt. basis 47% carbon, 7% hydrogen, 8% nitrogen, 30% oxygen, 0.1% phosphorus, 7% of other elements and a calculated HHV of 21.95 MJ/kg which demonstrated high potential for fuel production.

The moisture (15% wt.), mineral (ash 10% wt.), protein content (36% wt.), lipids (7.3% wt.) and carbohydrates (31% wt.) were measured. These proportions are related to the growth conditions, and lipid content could be increased by stressing the algae to nitrogen deprivation. Proteins have a high market value and could be directly sold after extraction, while lipids and carbohydrates could be used to generate fuels, such as biodiesel and ethanol, respectively.

Other characteristics identified in *Chlorella vulgaris* was its thick cell wall, so disruption methods were necessary to improve the efficiency of the extractions. Ultrasonication using an immersed probe gave a 2.5 time improvement over the control approach and revealed to be the best method

among those tested (manual grinding, chemical treatment, microwave and ultrasonic bath).

Knowing the characteristics of the strain used, the experiments moved to direct transesterification with supercritical ethanol, firstly in a batch reactor and then progressed to the flow reactor.

In the batch reactor it was possible to verify the influence of water content, temperature and reaction time on the transesterification reaction. The water does not impair the SC-EtOH transesterification route which is an advantage when compared to the traditional catalyst route (H_2SO_4), but a dewatering step of the algae is still necessary. The increase in temperature caused an increase in the biodiesel yield produced until the high temperature starts to decompose the feedstock (around 300°C). After understanding the direct transesterification process, a flow reactor apparatus was designed and constructed where a maximum yield of 47.5% wt. was achieved at 260°C, 75 bar, flow of 2 $mL \cdot min^{-1}$ for an algae concentration of 6 $mg \cdot mL^{-1}$ water.

The production of biodiesel from algae through direct transesterification with supercritical ethanol in the absence of a catalyst was made in a flow reactor and obtained promising results. The flow reactor showed capacity to improve the reaction when compared to the batch process (which only achieved 26% wt. yield) and is essential to the scale-up process, but it was not possible to identify the best conditions for the SC-EtOH transesterification due the instability of the system. Other interesting discovery in this process was the presence of phytol in the

biodiesel when created using the supercritical ethanol route (batch or flow) which needs to be further studied.

The proposed route (cultivation, dewatering and SC-EtOH transesterification) was found to be energetically feasible and is comparable to the base route (cultivation, dewatering, drying, oil extraction and transesterification with an acid catalyst) if an efficiency of 60% in the transesterification process is achieved. A similar observation was conducted for the environmental analysis where the new route is only competitive if the higher transesterification efficiency is achieved. These conclusions are based on an oil content of 20% wt., as the 7.3% wt. achieved in this work was at non-optimized conditions.

When compared to the soybean biodiesel route, the algae biodiesel presents environmental advantages, but has lower performance in the energy analysis. This highlights the importance in developing technologies to decrease the energy demand.

This study has demonstrated the potential of algae as feedstock for biodiesel production and has shown that it is possible to use supercritical ethanol for direct transesterification, thus eliminating the drying process and the use of a catalyst.

7.2. Future Work

The work undertaken was able to show possible locations for algae cultivation; however future work is necessary to collect more detailed geo-referenced data of climate, land use and CO_2 source in order to calculate the area with more accurately on a smaller scale.

The construction of pilot plants and industrial scale production is also necessary to provide data of maximum algae productivity and the operational problems faced at large scale. Also, the policies of each country and region also need to be periodically reviewed to include the application of feedstock diversification and new technologies.

It is important to develop methods to decrease the energy for cell disruption and protein extraction, since these are important steps for algae valorisation in biorefineries. It is also necessary to research the environment and media in which algae is cultivated in with the purpose of increasing lipid content which will be advantageous to process economics.

The combined supercritical ethanol extraction and transesterification process showed that it is possible to produce biodiesel without the need for separate drying and extraction steps. More research studies on the feasibility of this technology are required.

The flow reactor gave better results in terms of biodiesel yield than the batch reactor. A detailed analysis of the parameters involved to find the optimal conditions of the system is recommended.

The addition of CO_2 as a co-solvent in the transesterification batch process increased the yield. It is suggested that studies be undertaken to introduce CO_2 into the flow reactor. Other co-solvents could also be tested, such as hexane, which has already been utilised in batch a reactor in the literature.

The presence of phytol in the biodiesel product also needs to be further studied and how it could be utilised as a co-product. The biodiesel also needs to be produced in larger volumes in order to assess it against national and international biodiesel standards.

It is recommended that the supercritical fluid route is assessed with other algae strains and with the same strain with higher lipids content in order to measure their effect on process performance.

Once the direct transesterification technology in supercritical ethanol has been further developed and optimised, a new LCA analysis should be made in order to evaluate its feasibility, as the yield produced in this research was low in comparison to the traditional route and would not justify its use as an alternative route.

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Appendix 1 – LCA Main Works in Algae Biodiesel

Source	Feedstock	Boundary and FU	Assumptions	Technology	Results Units	Main Conclusions
(Lardon et al., 2009)	*Chlorella vulgaris*	"Well-to-wheel" 1 MJ of biodiesel	Facility covering 100 ha in Mediterranean context	Open raceways pond / continuous recirculation of culture ponds through a thickener and dewatering of the stream / oil extraction with hexane and direct extraction from the wet algal paste	Energy Demand, GWP, Ionizing radiation, Photochemical oxidation, marine toxicity, abiotic depletion, eutrophication, land use, acidification, human toxicity and ozone depletion	90% (70% with wet extraction) of the energy requirement is used in the lipid extraction stage / environmental impacts still high when compared to other feedstock / the biodiesel by algae needed more studies before to be feasible
(Jorquera et al., 2010)	*Nannochloropsis sp.*	Cultivation 100 t dry biomass	Data from USA	Raceway ponds, tubular and flat-plate photobioreactors	Net energy ratio (Energy produced / Energy requirements)	Raceway ponds and flat-plate photobioreactors revealed a NER>1 (different and more optimistic than other studies)
(Clarens et al., 2010)	Not specified	"Well-to-gate" 317 GJ of biomass energy	Objective of compare the biomass - conversion to biodiesel was excluded	Raceway open ponds with a combination of flocculation and centrifugation to harvesting	Land use, energy, GHG emissions, water and eutrophication	Algae biodiesel is more beneficial (environmentally) than the terrestrial crops (corn, switch grass and canola)
(Sander and Murthy, 2010)	Not specified	"Well-to-pump" 1,000 MJ of biodiesel	Data from other studies	Open pond and PBR / Chamber filter press or self-cleaning plate separator centrifuge / Natural gas fired dryer / Transport per 150 km / Hexane extraction and transesterification (same than soybean biodiesel plant)	Net energy ratio (Net energy input/Energy in functional unit), Net CO_2 (CO_2 demand emissions / CO_2 emissions in functional unit)	For each 1000 MJ of energy from algal biodiesel produced, 6,174 MJ of energy is required and the net CO_2 is -120.9
(Stephenson et al., 2010)	*Chlorella vulgaris*	"Well-to-wheel" 1 t biodiesel	Large-scale plant in UK with a capacity to produce 250,000 tons of biodiesel - modelling based in a small production	Cultivation in raceways and air-lift tubular bioreactors grown with carbon (as flue gas) from a gas-fired power station and the biodiesel production the same as the actual used in biodiesel from rapeseed	GWP	Raceways ponds have a GWP of proximally 80% lower than diesel, while the one cultivated in photobioreactor would increase the emissions

Source	Feedstock	Boundary and FU	Assumptions	Technology	Results Units	Main Conclusions
(Campbell et al., 2011)	Sea algae - *Botryococcus braunii* and *Dunaliella tertiolecta*	"Well-to-wheel" 1 t km	400 ha with ponds and more 100 ha with buildings, roads, etc with a production rate of 30g/m^2/d in Australia	Raceway open ponds, with CO_2 supplied by three routes (ammonia plant, as a flues gas (15% concentration) from a fossil-fuel power station and delivered by truck in liquefied form) / Air flotation system (hydrophobic polymer flocculant) / Centrifuge / Transesterification with methanol / Anaerobic digester for recycling the residual biomass	GWP	Algae biodiesel can reduce between 63.1 and 108.8 g/t/km and can be economically viable
(Yang et al., 2011)	*Chlorella vulgaris*	"Well-to-gate" 1 kg of biodiesel	Data from other studies. Some variations (specie, space, etc), a sensitive, analyses are done in the study for California (USA)	Open pond / harvesting / drying / extraction / esterification	Kg of water, nitrogen and phosphate	To produce 1 kg algae biodiesel, it is necessary 3726 kg water, 0.33 kg nitrogen and 0.71 kg phosphate without recycling the wastewater and less 84% in the water usage and 55% in nutrients with recycling
(Batan et al., 2010)	*Nannochloropsis sp.*	"Well-to-pump" 1 MJ of biodiesel	Pilot plant scale reactor system	Cultivation by open raceway ponds and photobioreactors / Dewatering by flocculation, centrifugation, vacuum belt dryers, or solar driers / Extraction by hexane with a shear mixer, centrifuge, decant tank, solvent recovery, and two distillation units for the recovery of solvents / Transesterification with methanol	Net Energy Ratio (energy consumed/fuel energy produced) and Net GHG emissions	Microalgae biodiesel process currently available in the market can have a NER of 0.93 and a Net GHG emissions of -75.29 gCO_2eq/MJ. Considering only this two parameters, it is better than soybean biodiesel
(Razon and Tan, 2011)	*Nannochloropsis sp.* and *Haematococcus pluvaris*	"Well-to-gate" 1 kg of biodiesel	Analysis of two different scenarios according to the feedstock	1. Flat-plate PBR and raceway pond. Thickener with microfilter, bead mill and decanter 2. Raceway Pond, thickener and dryer. Both end with transesterification with methanol and the process is also coupled with the biogas generation	Net Energy (Energy output/energy input)	There is a large energy deficit in the process due the high energy required during the cultivation and oil extraction, but the energy generation can be considered as by-product in a sewage treatment or in CCS plants
(Khoo et al., 2011)	*Nannochloropsis sp.*	"Well-to-gate" 1 MJ biofuel	Hypothetical lab-scale facility in Singapore	Cultivation in an integrated photobioreactor-raceway pond / Harvesting with $FeCl_3.6H_2O$ in an "air sparking assisted coagulation flocculation" (ASACF) / Extraction by hexane / Conversion by transesterification.	Energy and CO_2 balance	The total energy demands are 4.44 MJ (13% from biomass production, 85% from lipid extraction, and 2% from biodiesel production), but the author does not show the final CO_2 balance (just a graphic)

Source	Feedstock	Boundary and FU	Assumptions	Technology	Results Units	Main Conclusions
(Brentner et al., 2011)	*Scenedesmus dimorphus* (freshwater algae)	"Well-to-gate" 10 GJ biodiesel	Literature-based data and discussions from industry representatives (USA) / Not include labor, transport infrastructure, capital machinery, or combustion of the biodiesel	Five distinct processes (cultivation, harvesting and dewatering, oil extraction, conversion (transesterification), and byproduct management) which were sub divided in different options that could be settled in 160 pathways Cultivation: Raceway Pond; Annular, Tubular and Flat-Plate PBR Conversion: conventional transesterification, Supercritical CO_2 extraction, direct transesterification, supercritical methanol Harvesting: centrifugation, filtration, and flocculation/settling	Cumulative Energy Demand (MJeq); GHG emissions (kg CO_2eq); eutrophication (g Neq); direct water use (m3); cultivation land use (m2)	The best scenario was the usage of Flow-Plate PBR, Flocculation with chitosan, an extraction and conversion adding methanol in supercritical conditions and recycling the biomass in an anaerobic digestion
(Shirvani et al., 2011)	*Chlorella Vulgaris*	"Well-to-wheel" 1MJ of biodiesel	Facility with 0.1 ha and a water volume of 0.03 m^3/ha, productivity of 24.75 kg/day / Data from UK, France, Brazil, China, Nigeria and Saudi Arabia	Cultivation in open raceway ponds / harvesting (flocculant) and drying / dry extraction with hexane / transesterification / fuel distribution / combustion by end user	GHG emissions and energy balance ratio (EBR = Total fossil energy input/Total energy output)	Currently use 2.5 more energy than conventional diesel / Good solution to countries like Brazil and French that have a renewable energetic matrix.
(Clarens et al., 2010)	Salt-tolerant algae species (e.g., *Phaeodactylum sp.*, *Tetraselmis sp.*, etc.)	"Well-to-gate" 317 GJ of biomass	Assumed data from their earlier study in Southwestern USA	Four scenarios varying the conversion to biofuel stage and considering CO2 utilization, carbon capture from coal-fired power plant, flue gas usage and wastewater supplementation	Energy, GHG emissions and water	Depending on the specific combination of cultivation and conversion processes used, net energy can be positive or negative, so more LCA are necessaries
(Frank, 2011)	Not specified	"Well-to-wheels"	It is also produced renewable diesel and renewable gasoline via hydrotreating	Open Pond / Bioflocculation, DAF and Centrifuge / Hexane Extraction / Anaerobic Digestion	Energy, GHG emissions	The assumptions, such CO2 retained in algae, have low influence in the results of LCA, while parameters related to energy and nutrient recovery have largest consequences in direct emissions. The GHG emissions of algae biodiesel are lower than the conventional diesel, but algae biodiesel use more energy.

Source	Feedstock	Boundary and FU	Assumptions	Technology	Results Units	Main Conclusions
(Chowdhury et al., 2012)	*Schizochytrium limacinum* (different lipid contents were considerate)	"Well-to-gate"	Data collected from peer-reviewed publications and reports	Open raceway pond / Settling tanks / Extraction by hexane / Transesterification by methanol and acid catalyst / Anaerobic digestion to produce biogas	Energy Demand (NER - total fossil energy required/energy produced); GWP; Water Demand	Microalgae biofuels have the potential to be economically viable and environmentally sustainable. The integration with anaerobic digestion and nutrient recycling can decrease the external fossil energy inputs, the energy demand, global warming potential (GWP), and process water demand.
Vasudevan et al. (2012)	Not specified (Saline specie)	"Well-to-wheel"	4000 ha (40 ponds of 10 ha), producing 20 m3/ha/year. 3 scenarios: low-impact (optimistic), nominal, and high-impact (pessimistic)	Open Pond / Dissolved Air Flotation / Centrifuge Separation / 1. Bell Dryer and wet extraction and 2. Wet extraction / Algae oil transport / Refining /	GHG Emissions, water consumption (on site)	It is not possible to measure the viability of the process, because it is needed the development of the application of these technologies and scale-up the process. More GHG are emitted with dry extraction than wet extraction and secretion. The sustainability of the dry extraction is related to the source of energy used to dry the biomass. Saline systems that use brackish makeup water can consume the same amount of freshwater than conventional fuels
Delrue et al. (2012)	Not specified	"Well-to-gate"	Evaluate mix processes in the same pathway and use information from US and UK for France	1. Raceways, centrifugation, thermal drying, n-hexane lipid extraction, transesterification and anaerobic digestion 2. PBRs and raceways, belt filter press, DME lipid extraction, hydrotreating and anaerobic digestion.	NER; Production cost (€/L of biodiesel); GHG emission rate ($kgCO_2$-eq/100 km); Water consumption (L of water/L of biodiesel)	It is required the use of low-carbon energy sources to achieve significant reductions in GHG emission, besides the actual base scenario can be improved with environmental and energy use benefices
(Galindro, 2012)	*Nannochloropsis oculata*	Cultivation 1 kg of biomass	Lab-scale with effluent from shrimp production in Brazil	Raceway open pond	Acidification, eutrophication, GWP, Human toxic, Energy demand	The environmental impacts are lower using shrimp production effluent, but there are also nutrients that were not consumed in the medium
Ventura et al. (2013)	Not specified	"Well-to-gate"	Data from previous articles with cost and some CO_2 emissions information from Korean's sources	Scenario 1: biodiesel production Scenario 2: Scenario 1 + anaerobic digestion of the residuals after lipid extraction Scenario 3: biogas Scenario 4: mixed gas (supercritical gasification) production	energy, cost, and CO_2 analysis	The options proposed increase the net energy and CO_2 removal, but they remain expensive.

Source	Feedstock	Boundary and FU	Assumptions	Technology	Results Units	Main Conclusions
Sills et al. (2012)	Marine algae	"Well-to-wheels"	Coastal location in USA in a 1210 ha production facility with access to seawater / Focus on uncertainly	Cultivation in three levels of productivity (low, base and high) in geotextile-lined open raceway ponds and PBR / Harvesting and dewatering by in-pond sedimentation with autoflocculation and later centrifugation and a belt filter press / "Wet extraction" and "dry extraction" (hexane extraction and hydrothermal liquefaction - same than soybean) / Conversion by hidrotreatment and transesterification	Non-renewable energy demand, EROI (energy contained / total non-renewable energy required to produce) and GWP	EROI ratios from previous algal biofuel LCA studies varies from 0.09 to 4.3 / A role of uncertainty in the process was quantified by the extension of previous LCA studies / There are the necessity of developing viable wet lipid extraction technologies, incorporating high energy co-products, and reducing energy consumption during the cultivation
Borkowski et al. (2012)	Not specified	"Well-to-pump"	Compare the two technologies in identical cultivation and harvest conditions	Open pond cultivation with CO_2 injection by MEA and Flue gas / Transesterification with methanol and hydroxide and sodium methoxide as catalysts / Hydrotreating patented by UOP.	GHG (g CO_2-e/t km); Net Energy (MJ)	Renewable diesel has marginally lower energy intensity than biodiesel and greenhouse gas emissions are found to be almost identical.
Passell et al. (2013)	*Nannochloris sp.* and *Nannochloropsis sp.*	"Well-to-wheels"	Cultivation and harvesting data from a commercial algae producer (1000 m^2) and hypothetical scaled up facility (101,000 m^2), transport is not considered	Open pond / harvesting and dewatering with belt filter press / wet extraction with hexane / transesterification / combustion in a CIDI (compression-ignition direct-injection) vehicle	NER (energy in/energy out), GWP, photochemical oxidation potential, water depletion, particulate matter, total NOx and SOx	The productivity reported by the commercial producer is much lower than the one found in the bibliography (3 g/m^2/day against 20 to 30 g/m^2/day). The process efficiencies should be increased. It is necessary more LCAs to solve these divergences.
Torres et al. (2013)	Not specified (saline specie)	"Well-to-pump"	Pilot scale in Spain (3000 m^2 with a production of 40,000 metric tons (MT)/year)	Open pond / dynamic cross flow microfiltration and/or centrifugation and/or flocculation/sedimentation / wet extraction or dryer and dry extraction with hexane / alkaline transesterification with methanol / product purification	Total environmental impact in ecopoints (Eco-indicator 99)	Realistic scenarios were represented and the best route is using dynamic cross flow filtration, centrifugation and a dry extraction. Direct extraction has good economic results, the classic dry extraction is less damaging for the human health and ecosystem quality and the wet extraction has the highest environmental impacts.

Appendix 2 – BBM Algae – Growth Conditions

The algae cultivated at University of Birmingham are cultured by the School of Bioscience at approximately 24°C and 8000 lux. The incubation time is either 3 or 4 days with aeration to ensure mixing and prevent settlement of the algal cells. The cultures are established using 75 mL (for 4 days) or 100 mL (for 3 days) from a harvested culture added to new media up to 1750 mL. The algae are collected after 3 or 4 days of culture and then it can be used as harvested, concentrated or dried.

The BBM is prepared by adding to 3.5 L of dH_2O, 50 mL of stock solutions from 1 to 6, 5 mL from 7 to 9, 0.5 mL from 10 to 14 solutions. The total volume is completed with dH_2O to 5 litres. The pH is adjusted to 6.6 (but 6.4 to 6.8 are acceptable) with HCl (1M or 0.1M) or 1M NaOH. The stock solutions are prepared following the concentration shown in Table A1.1.

Table A2.1. BBM Stock Solutions

N°	Compound	Chemical Formula	Concentration (g/L)
1	Potassium di-hydrogen orthophosphate	KH_2PO_4	17.50
2	Di-potassium hydrogen orthophosphate	K_2HPO_4	7.50
3	Magnesium sulphate heptahydrate	$MgSO_4.7H_2O$	7.50
4	Sodium nitrate	$NaNO_3$	25.00
5	Calcium chloride dihydrate	$CaCl_2.2H_2O$	2.50
6	Sodium chloride	NaCl	2.50
7	EDTA tetrasodium salt	$EDTA\text{-}Na_4$	50.00
	+ Potassium hydroxide	KOH	31.00
8	Ferrous sulphate heptahydrate	$FeSO_4.7H_2O$	4.98
	+ conc. Sulphuric acid	H_2SO_4	10 mL/L
9	Boric acid	H_3BO_3	11.42
10	Zinc sulphate heptahydrate	$ZnSO_4.7H_2O$	14.12
11	Manganese chloride tetrahydrate	$MnCl_2.4H_2O$	2.32
12	Copper sulphate pentahydrate	$CuSO_4.5H_2O$	2.25
13	Cobaltous nitrate hexahydrate	$Co(NO_3)_2.6H_2O$	0.80
14	Sodium molybdate dihydrate	$Na_2MoO_4.2H_2O$	1.92

Appendix 3 – Gas Chromatography Calibration Curves

The calibration curves for FAME and FAEE for both GCs used (GC-2010 Plus Shimadzu and 6850 Agilent) are presented below.

A3.1. FAME calibration curves – Shimadzu

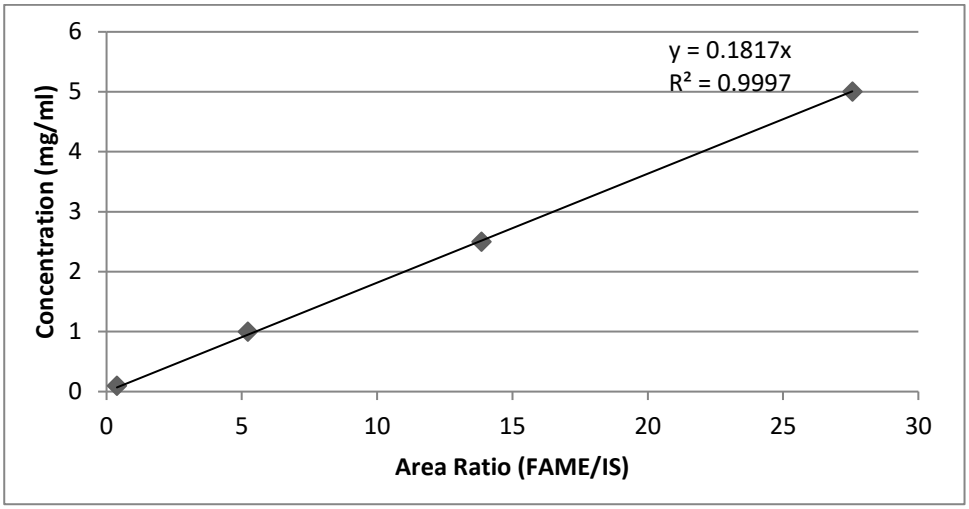


Figure A3.1. Palmitic ME calibration curve

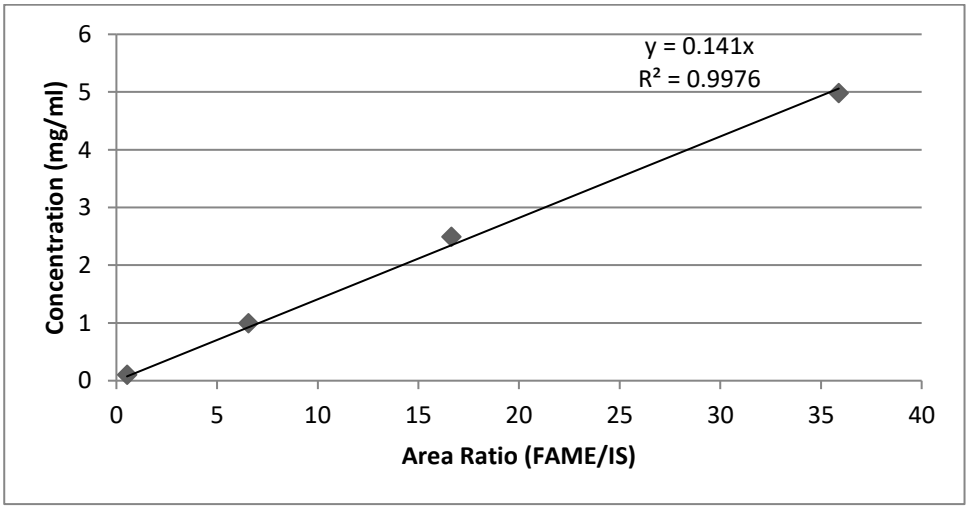


Figure A3.2. Oleic ME calibration curve

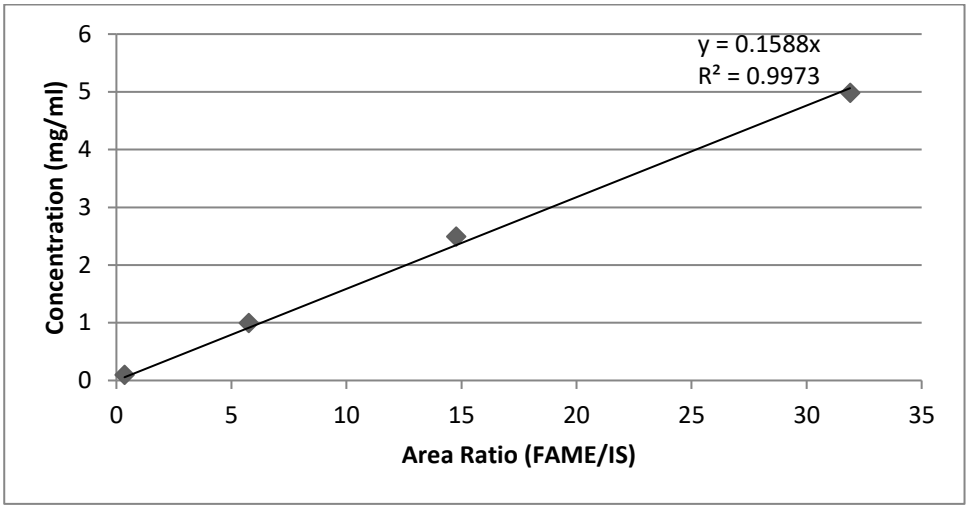


Figure A3.3. Linoleic ME calibration curve

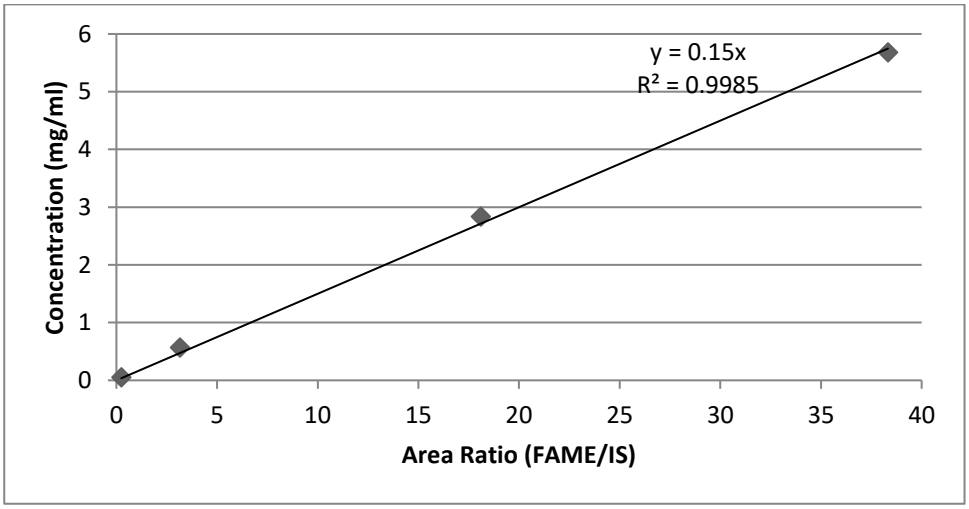


Figure A3.4. Linolenic ME calibration curve

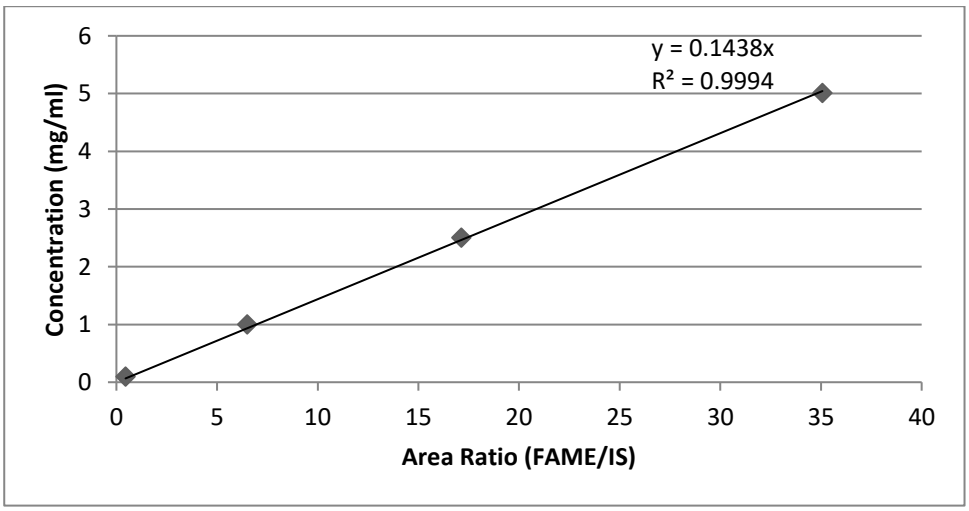


Figure A3.5. Stearic EE calibration curve

A3.2. FAEE calibration curves – Agilent

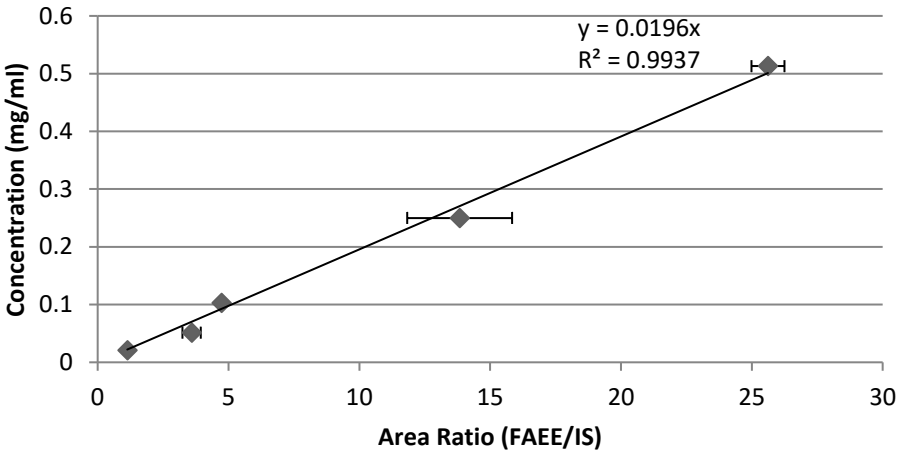


Figure A3.6. Palmitic EE calibration curve

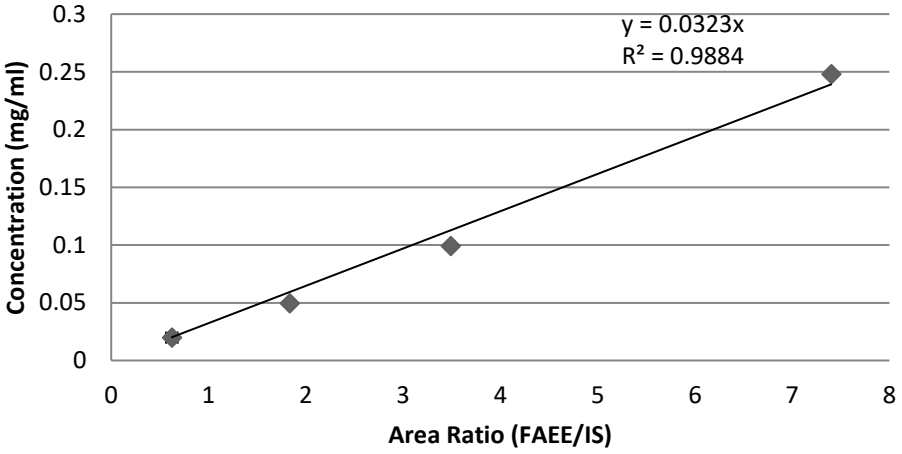


Figure A3.7. Oleic EE calibration curve

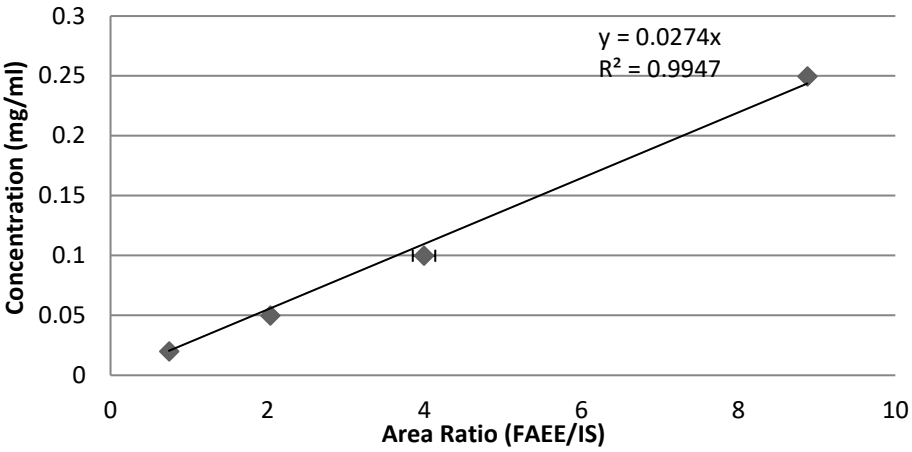


Figure A3.8. Linoleic EE calibration curve

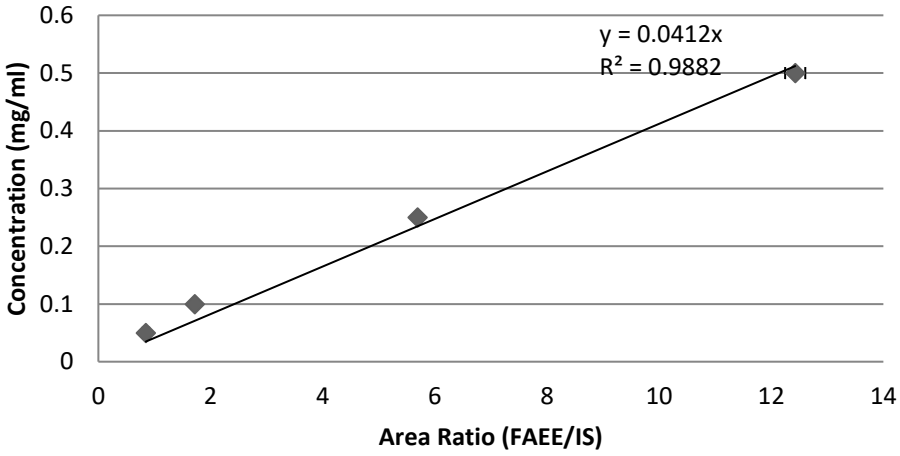


Figure A3.9. Linolenic EE calibration curve

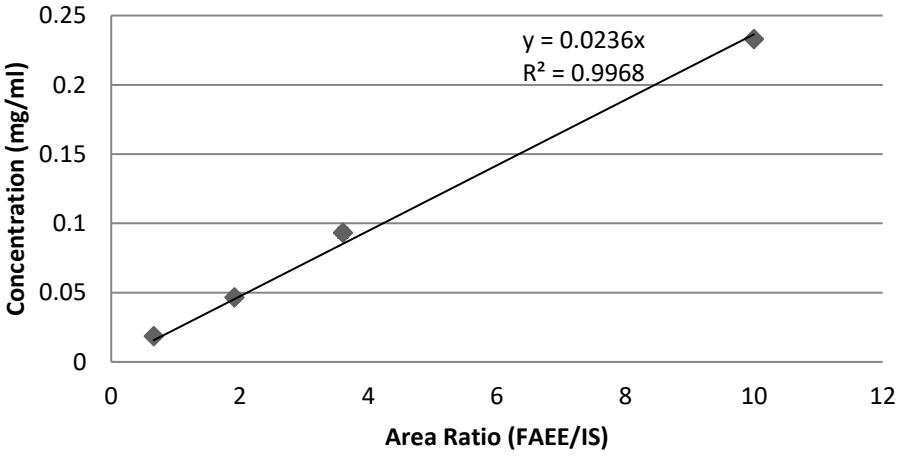
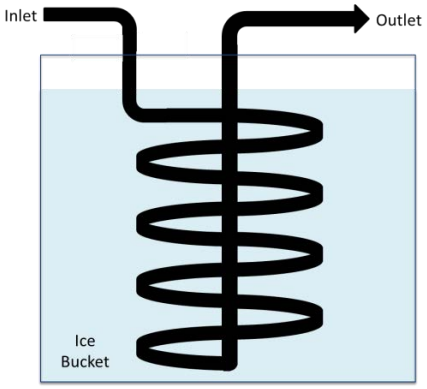


Figure A3.10. Stearic EE calibration curve

Appendix 4 – Cooling System Design

Material:

- Stainless steel 316 ¼ in. OD (ID = 0.46 cm)
- Ice bucket



Assumed data:

- Maximum flow: 10 mL/min
- Ethanol density at 20ºC: 0.789 g/mL
- Maximum initial temperature: 280°C
- Desired final temperature: 20°C

Calculations:

- Heat change (Q):
  - $Q = m\ C\ \Delta T$
  - Mass flow (m); specific heat (C); change in temperature (ΔT)
  - Q = 223.95 W
- Reynolds number (Re)
  - $Re = \rho\ u\ d\ \mu^{-1}$
  - Density (ρ); velocity of the fluid (u); hydraulic diameter (d); dynamic viscosity (μ)
  - Re = 1,608 -> Laminar flow
- Heat transfer coefficient of ethanol (h)
  - $h = Nu\ k'\ d^{-1}$
  - Nusselt number (Nu = 4.36 for laminar flow); hydraulic diameter (d); thermal conductivity of the fluid (k') [1]
  - $h = 122.15\ W/m^2K$
- Total heat transfer coefficient (U)
  - $U = (h^{-1} + (d_x\ k^{-1}))^{-1}$
  - Heat transfer coefficient of ethanol (h), thermal conductivity of the pipe (k) [2], Wall size (dx)
  - $U = 121.37\ W\ m^{-2}\ K^{-1}$
- Necessary length (L) based in the heat change in tubing (Q)
  - $Q = U\ L\ \pi\ d\ (\Delta Tlm)$
  - Heat (Q); change in temperature (ΔTlm); total heat transfer coef. (U)
  - L = 1.09 m
  - Add 30% extra (safety reasons): 1.42 m

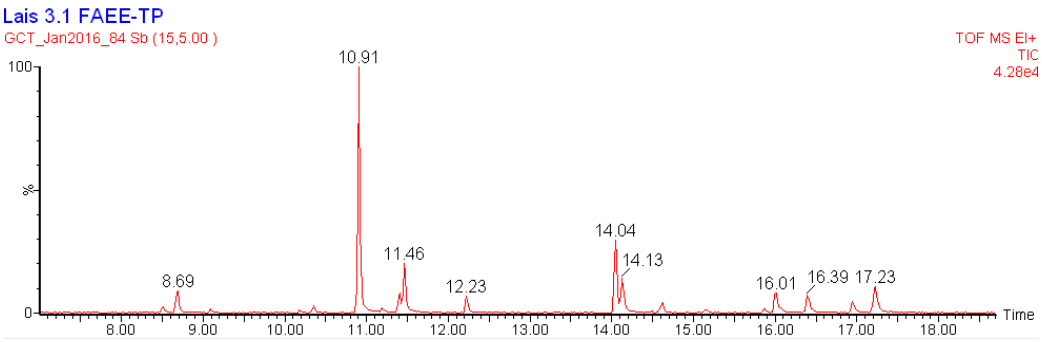
Colling system needs to have a minimum length of 1.42 m (representing 25 mL).

References:

[1] http://www.thermalfluidscentral.org/encyclopedia/index.php/Thermophysical_Properties:_Ethanol

[2] http://www.bssa.org.uk/topics.php?article=139

Appendix 5 – GC-MS



Time	Formula	Compound
8.51	$C_{17}H_{36}$	Heptadecane
8.69	$C_{17}H_{34}$	8-Heptadecene
9.09	$C_{17}H_{32}$	6,9-Heptadecadiene
10.91	$C_{20}H_{40}O$	Phytol
11.41	$C_{20}H_{40}O$	Phytol
11.46	$C_{20}H_{40}O$	Phytol
12.13	$C_{20}H_{40}O$	Phytol
14.04	$C_{18}H_{36}O_2$	Palmitic Acid Ethyl Ester
15.14	$C_{18}H_{36}O_2$	IS - Methyl Heptadecanoate
15.86	$C_{20}H_{40}O_2$	Stearic Acid Ethyl Ester
16.01	$C_{20}H_{38}O_2$	Oleic Acid Ethyl Ester
16.39	$C_{20}H_{36}O_2$	Linoleic Acid Ethyl Ester
16.94	$C_{20}H_{34}O_2$	Linolenic Acid Ethyl Ester
17.23	$C_{20}H_{40}O$	Phytol